PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 18 May 2001 (18.05.01)	
International application No. PCT/EP00/07023	**Applicant's or agent's file reference** 1999PTWO
International filing date (day/month/year) 21 July 2000 (21.07.00)	**Priority date** (day/month/year) 22 July 1999 (22.07.99)
Applicant MACCHIA, Bruno et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

19 February 2001 (19.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Nestor Santesso
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

AL MINISTERO DELL'INDUSTRIA DEL COMMERCIO E DELL'ARTIGIANATO

UFFICIO ITALIANO BREVETTI E MARCHI - ROMA

MODULO A

marca da bollo

DOMANDA DI BREVETTO PER INVENZIONE INDUSTRIALE, DEPOSITO RISERVE, ANTICIPATA ACCESSIBILITÀ AL PUBBLICO

A. RICHIEDENTE (I)

1) Denominazione: BRACCO S.P.A. — N.G. S.P

Residenza: MILANO — codice 00825120157

2) Denominazione:

Residenza: — codice

B. RAPPRESENTANTE DEL RICHIEDENTE PRESSO L'U.I.B.M.

cognome e nome: Livio Brighenti — cod. fiscale

denominazione studio di appartenenza: NOTARBARTOLO & GERVASI S.P.A.

via: Lungarno Amerigo Vespucci — n. 24 — città: Firenze — cap 50123 (prov) FI

C. DOMICILIO ELETTIVO destinatario

via: — n. — città: — cap — (prov)

D. TITOLO — classe proposta (sez/cl/scl) — gruppo/sottogruppo

Analoghi di ceramidi, processo per la loro preparazione e loro uso come anti-tumorali.

ANTICIPATA ACCESSIBILITÀ AL PUBBLICO: SI ☐ NO ☒ — SE ISTANZA: DATA ../../.. — N° PROTOCOLLO

E. INVENTORI DESIGNATI (cognome nome)

1) MACCHIA Bruno
2) BALSAMO Aldo
3) MACCHIA Marco
4) DEL TACCA Mario

F. PRIORITÀ

	nazione o organizzazione	numero di domanda	data di deposito	allegato S/R
1)			../../..	
2)			../../..	

per copia conforme all'originale
IL FUNZIONARIO

ESATTI DIRITTI DI SEGRETERIA

SCIOGLIMENTO RISERVE — Data — N° Protocollo

G. CENTRO ABILITATO DI RACCOLT ...ISMI, denominazione

H. ANNOTAZIONI SPECIALI

NESSUNA

DOCUMENTAZIONE ALLEGATA

	N. es.			
Doc. 1)	2	PROV	n. pag. 19	riassunto con disegno principale, descrizione e rivendicazioni (obbligatorio 1 esemplare)
Doc. 2)	0	PROV	n. tav.	disegno (obbligatorio se citato in descrizione, 1 esemplare
Doc. 3)	1	RIS		lettera d'incarico, procura o riferimento procura generale
Doc. 4)	0	RIS		designazione inventore
Doc. 5)	0	RIS		documenti di priorità con traduzione in italiano
Doc. 6)	0	RIS		autorizzazione o atto di cessione
Doc. 7)	0			nominativo completo del richiedente

SCIOGLIMENTO RISERVE — Data — N° Protocollo

confronta singole priorità

8) attestati di versamento, totale lire trecentosessantacinquemila= obbligatorio

COMPILATO IL 22 07 1999 — FIRMA DEL (I) RICHIEDENTE (I) Livio Brighenti

CONTINUA SUNO SI — NOTARBARTOLO & GERVASI S.P.A.

DEL PRESENTE ATTO SI RICHIEDE COPIA AUTENTICA SUNO SI

UFFICIO PROVINCIALE IND. COMM. ART. DI FIRENZE — codice 48

VERBALE DI DEPOSITO — NUMERO DI DOMANDA FI99A000169 — Reg. A

L'anno millenovecento novantanove, il giorno ventidue, del mese di luglio

Il (i) richiedente (i) sopraindicato (i) ha (hanno) presentato a me sottoscritto la presente domanda, corredata di n. 01 fogli aggiuntivi per la concessione del brevetto sopraripportato.

I. ANNOTAZIONI VARIE DELL'UFFICIO ROGANTE nessuna

IL DIRIGENTE — L'UFFICIALE ROGANTE

FOGLIO AGGIUNTIVO n. 01 di totali 0[redacted] DOMANDA N. REG. A [redacted]

AGGIUNTA MODULO A

D. RICHIEDENTE (I)

Denominazione / Residenza — N.G. — codice

Denominazione / Residenza — codice

Denominazione / Residenza — codice

Denominazione / Residenza — codice

Denominazione / Residenza — codice

Denominazione / Residenza — codice

E. INVENTORI DESIGNATI

	cognome nome		cognome nome
05	DANESI Romano		

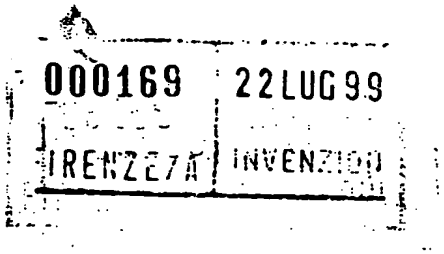
F. PRIORITÀ

nazione o organizzazione	tipo di priorità	numero di domanda	data di deposito	allegato S/R	SCIOGLIMENTO RISERVE Data	N° Protocollo

FIRMA DEL (I) RICHIEDENTE (I) Rino Rappelli

NOTARBARTOLO & GERVASI S.P.A.

SPAZIO RISERVATO ALL'UFFICIO ITALIANO BREVETTI E MARCHI



NOTARBARTOLO & GERVASI

322

BREVETTO

Stato	Ns. Rif.	Rif. Cliente	Class. Int.
ITALIA	1999PTIT		

Tipo Brevetto INVENZIONE INDUSTRIALE

Agente

Titolare **BRACCO S.P.A.**
Fatt. e Corr.: Via Folli, 50 20134 - MILANO

Inventore MACCHIA Bruno
BALSAMO Aldo
MACCHIA Marco
DEL TACCA Mario
DANESI Romano

Titolo Analoghi di ceramidi, processo per la loro preparazione e loro uso come antitumorali.

Data deposito		N. della domanda
22/07/1999		FI99A000169
Data rilascio	**Data visione pubblica**	**N. del brevetto**

Priorità

Durata anni	Dal	Al	Termine pagamento tasse
20	22/07/1999	22/07/2019	Dal 22/07/2002 ogni anno entro data dep.

Termine attuazione

3 anni dal rilascio o 4 anni dal deposito.

Note

MARCATURA: Consigliabile ma non obbligatoria.
TERMINE PER I DEPOSITI ALL'ESTERO CON RIVENDICAZIONE DI PRIORITA' 12 MESI DALLA DATA DI DEPOSITO
Pakistan, Taiwan, Ecuador, Tailandia, insieme ad altri Stati minori non aderiscono alla Convenzione di Parigi, di cui alleghiamo elenco degli Stati aderenti, e pertanto non riconoscono il diritto di priorità di cui all'art.4 della Convenzione. In questi Paesi è perciò opportuno effettuare il deposito il più presto possibile.

PROSPETTO A

RIASSUNTO INVENZIONE CON DISEGNO PRINCIPALE

NUMERO DOMANDA _____ REG. A DATA DI DEPOSITO __/__/____

NUMERO BREVETTO _____ DATA DI RILASCIO __/__/____

A. RICHIEDENTE (I)

Denominazione BRACCO S.P.A.

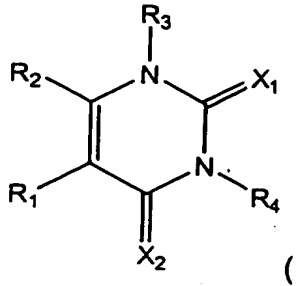
Residenza MILANO

D. TITOLO Analoghi di ceramidi, processo per la loro preparazione e loro uso come antitumorali.

Classe proposta (sez./cl./scl/) _____ (gruppo/sottogruppo) ____ ____

L. RIASSUNTO

Sono descritti i composti, analoghi di ceramidi, di formula (I):



(I)

Sono altresì descritti il processo di preparazione dei composti di formula (I), ed il loro uso per la preparazione di composizioni farmaceutiche per il trattamento di tumori.

M. DISEGNO

Descrizione dell'invenzione industriale dal titolo :

"Analoghi di ceramidi, processo per la loro preparazione e loro uso come antitumorali"

Titolare : BRACCO S.p.A.

con sede in : MILANO

Inventori designati : Bruno MACCHIA, Aldo BALSAMO, Marco MACCHIA, Mario DEL TACCA, Romano DANESI

Depositata il con il n°

* * * * *

CAMPO DELL'INVENZIONE

La presente invenzione riguarda i composti, analoghi di ceramidi, di formula generale (I) più avanti riportata, il relativo processo di preparazione, ed il loro uso per la preparazione di composizioni farmaceutiche ad azione antitumorale.

STATO DELLA TECNICA

Le ceramidi sono lipidi costituiti da un acido grasso e sfingosina legati tra loro da un legame ammidico, e sono generate dalla sfingomielina, uno sfingolipide presente nelle membrane di cellule eucariote, per azione dell'enzima sfingomielinasi, oppure sono sintetizzate per azione dell'enzima ceramide sintetasi.

Gli sfingolipidi come la sfingomielina sono stati sempre considerati come componenti strutturali, stabili e metabolicamente inattivi, delle membrane. Solo nell'ultimo decennio si è invece dimostrato che gli sfingolipidi hanno un ruolo attivo nei meccanismi di regolazione delle

risposte cellulari a stimoli esogeni, così come nella regolazione di crescita, differenziazione, trasformazione e adesione cellulare.

Si è inoltre recentemente dimostrato che i prodotti di demolizione degli sfingolipidi, quali ceramidi e sfingosina, giocano un ruolo importante nella regolazione dei meccanismi di trasmissione dei segnali controllati dagli sfingolipidi di membrana (Teruyuki Sakai et al., *Exp. Opin. Ther. Patents* (1998) 8 (12): 1673-1682). In particolare, la caratteristica distintiva di questi prodotti sembra essere la loro partecipazione ai meccanismi antiproliferativi di regolazione cellulare, quali ad esempio l'inibizione della crescita, l'induzione alla differenziazione e la morte programmata delle cellule, o apoptosi.

L'apoptosi è stata recentemente oggetto di numerosi studi (ad esempio Ross A. Kinloch et al., *TiPS*, Jan. 1999 (20): 35-42), dal momento che tale fenomeno si presta ad una "manipolazione" farmacologica: una diminuzione della frequenza di comparsa dell'apoptosi cellulare può infatti avere gravi conseguenze patologiche e facilitare la crescita di tumori, da cui il potenziale terapeutico di tutti quei composti che sono in grado di stimolare l'apoptosi.

Da studi approfonditi è risultato che le ceramidi presenti nelle membrane cellulari agiscono come "effettori" intracellulari dell'apoptosi, e quindi come potenziali inibitori della crescita dei tumori.

Al fine di incrementare farmacologicamente tale capacità delle ceramidi endogene, la strategia ottimale sembra essere quella di sviluppare analoghi delle ceramidi endogene che ne mimino gli effetti, che siano stabili nei confronti della metabolizzazione della ceramide a sfingosina e

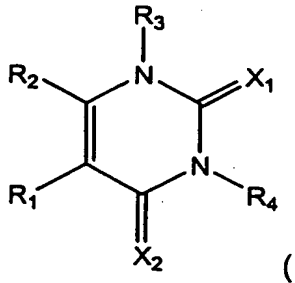
possiedano un'attività inibitoria sulla ceramidasi in modo da prevenire la generazione di sfingosina, che rappresenta un fattore di stimolazione della proliferazione, a partire dalle fonti endogene di ceramidi.

Tali analoghi delle ceramidi dovrebbero inoltre avere la capacità di penetrare le membrane cellulari.

E' pertanto sentita l'esigenza di disporre di composti analoghi di ceramidi, che siano in grado di attraversare le membrane cellulari, di penetrare all'interno delle cellule e di mimare le diverse proprietà delle ceramidi, in particolare quella di indurre l'apoptosi nelle cellule umane cancerose.

SOMMARIO

Ora la Richiedente ha sorprendentemente trovato che i composti analoghi di ceramidi di formula (I) :



(I)

in cui

X_1 e X_2 sono scelti tra O e S,

R_1 ed R_2 sono scelti tra $-(CH_2)_{13}CH_3$ e gruppi alchilici o alchilenici con da 2 a 6 atomi di carbonio, lineari o ramificati, non sostituiti o sostituiti con uno o più sostituenti scelti tra gruppi aromatici, amminici primari, secondari e terziari, ammonici quaternari, carbossilici, ossidrilici, eterei, atomi di alogeni o porzioni saccaridiche, con la condizione che uno solo tra R_1 e R_2 sia sempre $-(CH_2)_{13}CH_3$,

R_3 e R_4 sono scelti tra H e gruppi alchilici o alchilenici con da 2 a 6 atomi di carbonio, lineari o ramificati, non sostituiti o sostituiti con uno o più sostituenti scelti tra gruppi aromatici, amminici primari, secondari e terziari, ammonici quaternari, carbossilici, ossidrilici, eterei, atomi di alogeni o porzioni saccaridiche,

sono in grado di penetrare all'interno delle membrane biologiche ed indurre efficacemente l'apoptosi delle cellule cancerose.

I composti di formula generale (I) oggetto della presente invenzione sono quindi risultati adatti alla preparazione di composizioni farmaceutiche per il trattamento di tumori.

Rappresentano pertanto oggetto della presente invenzione i composti di formula generale (I), il relativo processo di preparazione ed il loro uso per la preparazione di composizioni farmaceutiche utili nel trattamento di tumori.

Le caratteristiche ed i vantaggi dei composti di formula generale (I) secondo la presente invenzione saranno illustrati in dettaglio nella seguente descrizione.

DESCRIZIONE DETTAGLIATA DELL'INVENZIONE

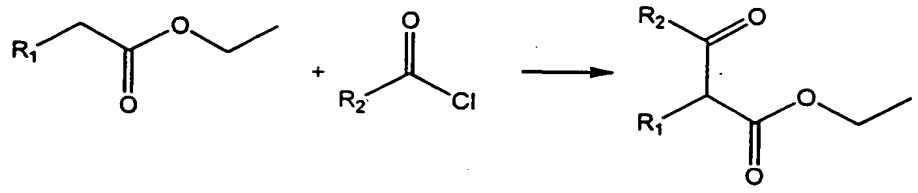
La presente invenzione si riferisce ai composti di formula generale (I) come sopra definiti. Tali composti (I) si sono rivelati in grado di penetrare all'interno delle membrane biologiche ed indurre efficacemente l'apoptosi delle cellule cancerose. Particolarmente efficaci e con una alta citotossicità sono risultati i seguenti composti:

- composto di formula (I) in cui X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = etile, e R_3 = R_4 = H [composto (3)].

- composto di formula (I) in cui $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = etile, e $R_3 = R_4 = H$ [composto (**4**)].
- composto di formula (I) in cui $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = n-propile, e $R_3 = R_4 = H$ [composto (**6**)].
- composto di formula (I) in cui $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = i-butile, e $R_3 = R_4 = H$ [composto (**10**)].

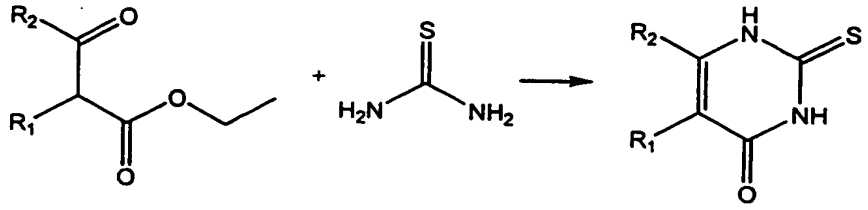
Il processo di preparazione dei presenti composti di formula (I) in cui $R_3 = R_4 = H$ comprende i seguenti stadi:

i) reazione dell'etil estere (II) con il cloruro acilico (III) per ottenere il β-chetoestere di formula (IV):



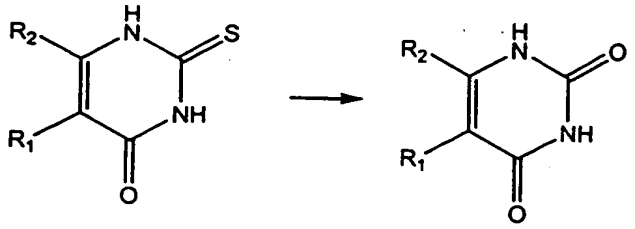
(II) (III) (IV)

ii) reazione del β-chetoestere di formula (IV) con tiourea (V) per ottenere il composto di formula (I) in cui X_1 è S e X_2 è O:



(IV) (V) (I)

iii) reazione del composto di formula (I) in cui X_1 è S e X_2 è O con acido cloroacetico a riflusso per ottenere il composto di formula (I) in cui $X_1 = X_2 = O$:



(I) (I)

dove X_1, X_2, R_1 e R_2 hanno i significati sopra indicati.

Lo stadio i) del presente processo è generalmente condotto in un solvente organico, ad esempio THF, alla temperatura di 0°C. Tale reazione è preferibilmente condotta in atmosfera inerte.

Il prodotto di reazione di formula (IV) può essere recuperato dalla miscela di reazione per aggiunta di una soluzione satura di NH_4Cl e successiva estrazione con etere etilico.

Lo stadio ii) del presente processo è condotto mediante aggiunta di tiourea in etanolo ed etossido di sodio, sul prodotto grezzo di reazione proveniente dallo stadio i), senza bisogno di effettuare alcuna purificazione. Allo stadio ii) la temperatura è mantenuta preferibilmente attorno ai 90°C. Il prodotto di reazione viene generalmente recuperato dalla miscela di reazione per acidificazione a pH 2, ad esempio per aggiunta di HCl conc., e filtrazione del precipitato formatosi, che può essere eventualmente purificato mediante lavaggi con acetone.

Il prodotto di reazione ottenuto allo stadio ii) può essere ulteriormente purificato mediante cromatografia su gel di silice, preferibilmente utilizzando come eluente la miscela acetato di etile : etere di petrolio in rapporto 2 : 1.

Lo stadio iii) del processo secondo la presente invenzione viene generalmente condotto aggiungendo al prodotto proveniente dallo stadio

ii) acido cloroacetico, ad esempio nella forma di soluzione acquosa al 10%, e scaldando a riflusso. Il residuo grezzo così ottenuto può essere purificato mediante lavaggio con etanolo assoluto e poi con etere etilico.

Il prodotto proveniente dallo stadio iii) può essere ulteriormente purificato mediante cromatografia su gel di silice, preferibilmente utilizzando come eluente la miscela acetato di etile : esano in rapporto 1 : 2.

I presenti composti di formula (I) in cui R_3 e/o R_4 sono diversi da H possono essere preparati a partire dal β-chetoestere di formula (IV) o dai composti di formula (I) in cui $R_3 = R_4 = H$, ottenuti come sopra descritto, mediante processi noti.

I composti di formula (I) secondo la presente invenzione possono essere formulati con eccipienti e/o diluenti farmaceuticamente accettabili, allo scopo di preparare composizioni farmaceutiche atte al trattamento delle patologie tumorali.

I seguenti esempi sono riportati a scopo illustrativo, ma non limitativo della presente invenzione.

ESEMPIO 1

Preparazione del composto di formula (I) in cui $X_1 = S$, $X_2 = O$, $R_2 = -(CH_2)_{13}CH_3$, R_1 = etile, e $R_3 = R_4 = H$ (1)

Una soluzione preparata sciogliendo 0,37 g di etil butirrato (3.17 mmoli) in 2 ml di THF anidro viene addizionata goccia a goccia, alla temperatura di 0°C e sotto atmosfera di argon, a 1,9 ml di una soluzione 2M di litio diisopropilammide (LDA) in THF anidro. Dopo 30 minuti di agitazione a 0°C, la miscela di reazione viene addizionata ad di una soluzione ottenuta sciogliendo 1 g di pentadecanoil cloruro (3,8 mmoli) in 5 ml di

THF anidro, precedentemente raffreddata a 0°C. La miscela risultante è mantenuta sotto agitazione a temperatura ambiente per 12 ore, e quindi addizionata di una soluzione satura di NH_4Cl. Si separa la fase organica da quella acquosa, che viene poi estratta con etere etilico. Gli estratti organici vengono riuniti, lavati con soluzione acquosa satura di NaCl, seccati con Na_2SO_4 anidro e quindi evaporati a secchezza per fornire un residuo grezzo (1,20 g) costituito quasi esclusivamente dal β-chetoestere (IV) in cui R_2 = $-(CH_2)_{13}CH_3$ e R_1 = etile. [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.83-0.94 (m, 6H), 1.07 (t, 3H, *J* = 7.4 Hz), 1.15-1.36 (m, 24H), 1.81-2.02 (m, 2H), 2.11-2.57 (m, 2H), 3.34 (t, 1H, *J* = 7.3 Hz), 4.15 (q, 2H, *J* = 7.3 Hz). MS m/e 340 M^+].

Il residuo grezzo (1,20 g) contenente il β-chetoestere (IV) in cui R_2 = $-(CH_2)_{13}CH_3$ e R_1 = etile, così ottenuto, viene disciolto in 20 ml di etanolo assoluto e quindi addizionato con 3,61 g di tiourea (47.5 mmoli) e 6,47 g di sodio etossido (95.1 mmoli). La miscela risultante è mantenuta sotto agitazione per 1 ora a 90°C. Dopo raffreddamento fino a temperatura ambiente, la miscela di reazione è filtrata ed il filtrato è evaporato a secchezza; il residuo così ottenuto viene quindi ripreso con una miscela acqua : THF in rapporto 10 : 1 fino a completa solubilizzazione. La soluzione, raffreddata a 0°C, è acidificata a pH 2 con HCl conc.; il precipitato formatosi, filtrato e lavato con piccole quantità di acetone, fornisce un residuo grezzo che viene purificato per cromatografia su gel di silice eluendo con acetato di etile : etere di petrolio in rapporto 2 : 1, ottenendo infine 290 mg (0,82 mmol; resa = 26%) del composto desiderato di formula (I) (p.f. = 167-169°C; ^{1}H-NMR ($CDCl_3$, 200MHz) δ

0.89 (t, 3H, J = 6.2 Hz), 1.09 (t, 3H, J = 7.4 Hz), 1.17-1.36 (m, 24H), 2.34-2.49 (m, 4H), 8.88 (br, 1H, D_2O scambiabile), 9.81 (br, 1H, D_2O scambiabile); MS m/e 352 M^+).

ESEMPIO 2

Preparazione del composto di formula (I) in cui $X_1 = X_2 = O$, $R_2 = -(CH_2)_{13}CH_3$, R_1 = etile, e $R_3 = R_4 = H$ (**2**)

160 mg (0,45 mmol) del prodotto (**1**) ottenuto come descritto nell'Esempio 1 vengono addizionati di 11,4 ml di una soluzione acquosa di acido cloroacetico al 10%, e la miscela così ottenuta è riscaldata per 12 ore a riflusso. Il precipitato risultante è quindi filtrato, lavato con etanolo assoluto e successivamente con etere etilico, per ottenere un residuo grezzo che, purificato mediante cromatografia su gel di silice, usando come eluente una miscela acetato di etile : esano in rapporto 1 : 2 ha permesso di ottenere 48 mg (0,14 mmol, resa = 32%) del composto desiderato puro (p.f. 132-134°C; ^{1}H-NMR ($CDCl_3$, 200MHz) δ 0.87 (t, 3H, J = 6.2 Hz), 1.06 (t, 3H, J = 7.4 Hz), 1.15-1.36 (m, 24H), 2.31-2.49 (m, 4H), 9.06 (br, 1H, D_2O scambiabile), 9.89 (br, 1H, D_2O scambiabile); MS m/e 336 M^+).

ESEMPIO 3

Preparazione del composto di formula (I) in cui $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = etile, e $R_3 = R_4 = H$ (**3**)

Una soluzione ottenuta sciogliendo 1 g di etil palmitato (3.52 mmoli) in 3 ml di THF anidro viene aggiunta goccia a goccia, alla temperatura di 0°C e sotto atmosfera di argon, a 2,1 ml di una soluzione 2M di litio diisopropilammide (LDA) in THF anidro. Dopo 30 minuti di agitazione alla

temperatura di 0°C, la miscela di reazione è aggiunta ad una soluzione ottenuta sciogliendo 2,39 g di propionil cloruro (4.23 mmoli) in 5 ml di THF anidro. La miscela risultante è mantenuta sotto agitazione a temperatura ambiente per 12 ore e quindi addizionata con una soluzione satura di NH_4Cl. La fase organica viene separata da quella acquosa, che è quindi estratta con etere etilico. Gli estratti organici vengono riuniti, lavati con soluzione acquosa satura di NaCl, seccati su Na_2SO_4 anidro e quindi evaporati a secchezza per fornire 1,31 g di un residuo grezzo costituito quasi esclusivamente dal β-chetoestere (IV) in cui R_1 = -$(CH_2)_{13}CH_3$ e R_2 = etile. [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.79-0.92 (m, 6H), 1.11 (t, 3H, *J* = 7.6 Hz), 1.17-1.39 (m, 24H), 1.48-1.62 (m, 2H), 2.26 (q, 2H, *J* = 7.6 Hz), 3.36 (t, 1H, *J* = 7.3 Hz), 4.15 (q, 2H, *J* = 7.2 Hz) ; MS m/e 340 M$^+$].

1,31 g del residuo grezzo contenente il β-chetoestere (IV) in cui R_1 = -$(CH_2)_{13}CH_3$ e R_2 = etile, così ottenuto, vengono sciolti in 20 ml di etanolo assoluto, e alla soluzione così ottenuta vengono aggiunti 4,01 g di tiourea (52,8 mmoli) e 7,18 g di sodio etossido (105,6 mmoli). La miscela risultante è mantenuta sotto agitazione per 1 ora a 90°C. Dopo raffreddamento fino a temperatura ambiente, la miscela di reazione è filtrata ed il filtrato viene evaporato a secco ; il residuo così ottenuto viene quindi trattato con una miscela di acqua : THF in rapporto 10 : 1 fino a completa solubilizzazione. La soluzione ottenuta, raffreddata a 0°C, è portata a pH 2 con HCl conc. ; il precipitato formatosi per acidificazione, filtrato e lavato con piccole quantità di acetone, consente di ottenere un residuo grezzo che, purificato per cromatografia su gel di

silice usando come eluente la miscela acetato di etile : etere di petrolio in rapporto 2 : 1, fornisce 310 mg (0,88 mmol ; resa = 25%) di un prodotto che risulta essere il composto desiderato 3 puro (p.f. 100-102°C ; ^{1}H-NMR ($CDCl_3$, 200MHz) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.01 (t, 3H, *J* = 7.4 Hz), 1.18-1.38 (m, 24H), 2.35 (t, 2H, *J* = 7.4 Hz), 2.48 (q, 2H, *J* = 7.6 Hz), 9.08 (br, 1H, D_2O scambiabile), 9.73 (br, 1H, D_2O scambiabile) ; MS m/e 352 M^+).

ESEMPIO 4

Preparazione del composto di formula (I) in cui X_1 = X_2 = O, R_1 = -$(CH_2)_{13}CH_3$, R_2 = etile, e R_3 = R_4 = H (4)

A 160 mg (0,45 mmol) del composto (3) ottenuto come descritto nell'Esempio 3 vengono aggiunti 11,4 ml di una soluzione acquosa di acido cloroacetico al 10%, e la miscela così ottenuta è riscaldata per 12 ore a riflusso. Il precipitato risultante viene filtrato, lavato prima con etanolo assoluto, quindi con etere etilico, per ottenere un residuo grezzo che, purificato per cromatografia su gel di silice, eluendo con una miscela acetato di etile : esano in rapporto 1 : 2, consente di ottenere 57 mg (0,17 mmol ; resa = 38%) del composto (4) (p.f. 110-112°C ; ^{1}H-NMR ($GDCl_3$, 200MHz) δ 0.89 (t, 3H, *J* = 6.4 Hz), 1.02 (t, 3H, *J* = 7.4 Hz), 1.12-1.42 (m, 24H), 2.34 (t, 2H, *J* = 7.2 Hz), 2.49 (q, 2H, *J* = 7.6 Hz), 9.15 (br, 1H, D_2O scambiabile), 9.53 (br, 1H, D_2O scambiabile) ; MS m/e 336 M^+).

ESEMPIO 5

Preparazione del composto di formula (I) in cui X_1 = S, X_2 = O, R_1 = -$(CH_2)_{13}CH_3$, R_2 = n-propile, e R_3 = R_4 = H (5)

Il composto (**5**) è stato preparato seguendo una procedura analoga a quella descritta nell'Esempio 3, ottenendo un prodotto per il quale è risultato: MS m/e 366 M^+.

ESEMPIO 6

Preparazione del composto di formula (I) in cui $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = n-propile, e $R_3 = R_4 = H$ (**6**)

Il composto (**6**) è stato preparato seguendo una procedura analoga a quella descritta nell'Esempio 4, ottenendo un prodotto per il quale è risultato: MS m/e 350 M^+.

ESEMPIO 7

Preparazione del composto di formula (I) in cui $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = n-butile, e $R_3 = R_4 = H$ (**7**)

Il composto (**7**) è stato preparato seguendo una procedura analoga a quella descritta nell'Esempio 3, ottenendo un prodotto per il quale è risultato: MS m/e 380 M^+.

ESEMPIO 8

Preparazione del composto di formula (I) in cui $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = n-butile, e $R_3 = R_4 = H$ (**8**)

Il composto (**8**) è stato preparato seguendo una procedura analoga a quella descritta nell'Esempio 4, ottenendo un prodotto per il quale è risultato: MS m/e 364 M^+.

ESEMPIO 9

Preparazione del composto di formula (I) in cui $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = i-butile, e $R_3 = R_4 = H$ (**9**)

Il composto (**9**) è stato preparato seguendo una procedura analoga a quella descritta nell'Esempio 3, ottenendo un prodotto per il quale è risultato: MS m/e 380 M^+.

ESEMPIO 10

Preparazione del composto di formula (I) in cui $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = i-butile, e $R_3 = R_4 = H$ (**10**)

Il composto (**10**) è stato preparato seguendo una procedura analoga a quella descritta nell'Esempio 4, ottenendo un prodotto per il quale è risultato: MS m/e 364 M^+.

ESEMPIO 11

Preparazione del composto di formula (I) in cui $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = neopentile, e $R_3 = R_4 = H$ (**11**)

Il composto (**11**) è stato preparato seguendo una procedura analoga a quella descritta nell'Esempio 3, ottenendo un prodotto per il quale è risultato: MS m/e 394 M^+.

ESEMPIO 12

Preparazione del composto di formula (I) in cui $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = neopentile, e $R_3 = R_4 = H$ (**12**)

Il composto (**12**) è stato preparato seguendo una procedura analoga a quella descritta nell'Esempio 4, ottenendo un prodotto per il quale è risultato: MS m/e 378 M^+.

ESEMPIO 13

Preparazione del composto di formula (I) in cui $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = 2-fenil-etile, e $R_3 = R_4 = H$ (**13**)

Il composto (**13**) è stato preparato seguendo una procedura analoga a quella descritta nell'Esempio 3, ottenendo un prodotto per il quale è risultato: MS m/e 428 M^+.

ESEMPIO 14

Preparazione del composto di formula (I) in cui $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = 2-fenil-etile, e $R_3 = R_4 = H$ (**14**)

Il composto (**14**) è stato preparato seguendo una procedura analoga a quella descritta nell'Esempio 4, ottenendo un prodotto per il quale è risultato: MS m/e 412 M^+.

TEST DI CITOTOSSICITA'

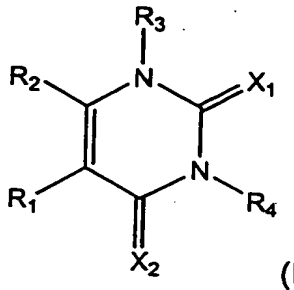
La citotossicità dei composti sintetizzati come descritto negli Esempi 1-14 è stata valutata utilizzando una linea cellulare di leucemia umana denominata CCRF/CEM. Le cellule CCRF/CEM sono state coltivate in mezzo di coltura contenente RPMI 1640 (90%), siero fetale bovino (10%) e interleuchina 2 (100 U/ml). Il saggio di citotossicità è stato effettuato su 104 cellule CCRF/CEM seminate in pozzetti da 35 mm in 2 ml di mezzo di coltura. Le cellule sono state trattate per 72 ore con i composti in esame e al termine del periodo di esposizione il loro numero è stato misurato e confrontato con quello di cellule di controllo trattate con C_2-ceramide al fine di determinare la percentuale di inibizione della crescita. La concentrazione in grado di inibire il 50% della crescita cellulare è stata calcolata per regressione non lineare dei dati sperimentali come descritto in M. Macchia, N. Jannitti, G.B. Gervasi, R. Danesi, *J. Med. Chem.*, (1996) 39 (7): 1352-1356.

I valori di IC_{50} espressi in μM così ottenuti sono riportati nella seguente Tabella:

Composto	IC_{50} (μM)
controllo	31,6
(3)	1,7
(4)	6,3
(6)	0,97
(9)	13,2
(10)	8,7
(11)	20
(12)	29,1
(13)	20,7
(14)	15,6

RIVENDICAZIONI

1. Composti di formula generale (I) :



(I)

in cui

X_1 e X_2 sono scelti tra O e S;

R_1 ed R_2 sono scelti tra $-(CH_2)_{13}CH_3$ e gruppi alchilici o alchilenici con da 2 a 6 atomi di carbonio, lineari o ramificati, non sostituiti o sostituiti con uno o più sostituenti scelti tra gruppi aromatici, amminici primari, secondari e terziari, ammonici quaternari, carbossilici, ossidrilici, eterei, atomi di alogeni o porzioni saccaridiche, con la condizione che uno solo tra R_1 ed R_2 sia sempre $-(CH_2)_{13}CH_3$;

e R_3 e R_4 sono scelti tra H e gruppi alchilici o alchilenici con da 2 a 6 atomi di carbonio, lineari o ramificati, non sostituiti o sostituiti con uno o più sostituenti scelti tra gruppi aromatici, amminici primari, secondari e terziari, ammonici quaternari, carbossilici, ossidrilici, eterei, atomi di alogeni o porzioni saccaridiche.

2. I composti di formula generale (I) secondo la rivendicazione 1, in cui:

a) X_1 = S, X_2 = O, R_1 = etile, R_2 = $-(CH_2)_{13}CH_3$, e R_3 = R_4 = H [composto (1)];

b) X_1 = X_2 = O, R_1 = etile, R_2 = $-(CH_2)_{13}CH_3$, e R_3 = R_4 = H [composto (2)];

c) X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = etile, e R_3 = R_4 = H [composto (3)];

d) $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = etile, e $R_3 = R_4 = H$ [composto (**4**)];

e) $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = n-propile, e $R_3 = R_4 = H$ [composto (**5**)];

f) $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = n-propile, e $R_3 = R_4 = H$ [composto (**6**)];

g) $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = n-butile, e $R_3 = R_4 = H$ [composto (**7**)];

h) $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = n-butile, e $R_3 = R_4 = H$ [composto (**8**)];

i) $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = i-butile, e $R_3 = R_4 = H$ [composto (**9**)];

l) $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = i-butile, e $R_3 = R_4 = H$ [composto (**10**)];

m) $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = neopentile, e $R_3 = R_4 = H$ [composto (**11**)];

n) $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = neopentile, e $R_3 = R_4 = H$ [composto (**12**)];

o) $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = 2-fenil-etile, e $R_3 = R_4 = H$ [composto (**13**)];

p) $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = 2-fenil-etile, e $R_3 = R_4 = H$ [composto (**14**)].

3. Processo per la preparazione dei composti di formula (I) in cui $R_3 = R_4 = H$ descritti nelle rivendicazioni 1 e 2, detto processo comprendendo i seguenti stadi :

i) reazione dell'etil estere (II) con il cloruro acilico (III) per ottenere il β-chetoestere di formula (IV);

ii) reazione del β-chetoestere di formula (IV) con tiourea (V) per ottenere il composto di formula (I) in cui X_1 è S e X_2 è O;

iii) reazione del composto di formula (I) in cui X_1 è S e X_2 è O con acido cloroacetico a riflusso per ottenere il composto di formula (I) in cui X_1 = X_2 = O,

dove X_1, X_2, R_1 e R_2 hanno i significati indicati nelle rivendicazioni 1 e 2.

4. Composizioni farmaceutiche comprendenti come principio attivo una almeno uno dei composti di formula generale (I) descritti nelle rivendicazioni 1 e 2, e/o loro derivati o sali farmaceuticamente accettabili, insieme ad eccipienti e/o diluenti.

5. Uso dei composti di formula generale (I) descritti nelle rivendicazioni 1 e 2 per la preparazione di composizioni farmaceutiche utili nel trattamento dei tumori.

(BRA)

Firenze, 21 Luglio 1999

p. BRACCO S.p.A.

il Mandatario

Dr. Livio Brighenti

della NOTARBARTOLO & GERVASI

NOTARBARTOLO & GERVASI

BREVETTO

Stato/Country	Ns. Rif./Our ref.	Rif. Cliente/Yr. ref.	Class. Int./Int. Class.
PCT (*)	1999PTWO		

Tipo Brevetto Patent	INVENZIONE INDUSTRIALE
Agente Agent	NOTARBARTOLO & GERVASI
Titolare Applicant	**BRACCO S.P.A.** fatt. e corr.: Via Egidio Folli, 50 - 20134 MILANO
Inventore Inventor	MACCHIA Bruno, BALSAMO Aldo, MACCHIA Marco, DEL TACCA Mario, DANESI Romano
Titolo Title	**CERAMIDE ANALOGS, PROCESS FOR THEIR PREPARATION AND THEIR USE AS ANTITUMOR AGENTS**

Data deposito/Filing date 21 Luglio 2000		**N. della domanda/Appln. No.** PCT/EP00/07023
Data rilascio/Granting date	**Data visione pubblica/Publication**	**N. del brevetto/Patent No.**

Priorità/Priority ITALIA - Domanda n. FI99A000169 del 22.7.1999

Durata anni/Duration	Dal/From	Al/To	Termine pagamento tasse Payment of fees

Termine attuazione/Working nazionalizzazione: 20 mesi dalla priorità; se si richiede esame preliminare, il termine è procrastinato a 30 mesi

Note/Notes

Entro 20 mesi (o 30 mesi) dalla data di priorità occorrerà provvedere alla nazionalizzazione nei Paesi designati

(*) Paesi designati: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH/LI, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, GW, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AP, EA, EP, OA

EPA / EPO / OEB
D - 80298 München
☎ 089 / 2399 - 0
Tx 523 656 epmu d
Fax 089 / 2399 - 4465

Europäisches Patentamt | European Patent Office | Office européen des brevets

EPA / EPO / OEB : D-80298 München

Gervasi Gemma
Notarbartolo & Gervasi S.p.A.
Corso die Porta Vittoria 9

20122 Milano

Nr. der Anmeldung / Application No. / Demande de brevet no PCT/EP 00 / 07023
Tag des Eingangs / Date of receipt / Date de réception 21. Juli 2000
Zeichen des Anmelders / Vertreter - Applicant / Representative ref. no. - Référence du demandeur ou du mandataire 1999PTWO

Anmelder / Applicant / Demandeur : Bracco S.p.A.

Datum / Date 22.07.00

Empfangsbescheinigung / Receipt for documents / Récépissé de documents

Das Europäische Patentamt bescheinigt hiermit den Empfang folgender Dokumente:
The European Patent Office hereby acknowledges the receipt of the following:
L'Office européen des brevets accuse réception des documents indiqués ci-dessous:

A. Internationale Anmeldung / International application / Demande internationale

		Stückzahl / No. of copies / Nombre d'exemplaires
[x]	Antrag / Request / Requête	1
[x]	Beschreibung (ohne Sequenzprotokollteil) Description (excluding sequence listing part) Description (sauf partie réservée au listage des séquences	3
[x]	Patentansprüche / Claim(s) / Revendication(s)	3
[x]	Zusammenfassung / Abstract / Abrégé	1
[x]	Zeichnung(en) / Drawing(s) / Dessin(s)	
[ ]	Sequenzprotokollteil der Beschreibung Sequence listing part of description Partie de la description réservée au listage des séquences	
[ ]	Diskette / Disquette	

[x]	Kopie der allgemeinen Vollmacht Copy of general power of attorney Copie du povoir général	
[x]	Prioritätsbeleg(e) Priority document(s) Document(s) de priorité	
[ ]	Blatt für die Gebührenberechnung Fee calculation sheet Feuille de calcul des taxes	
[ ]	Abbuchungsauftrag Debit order Ordre de débit	Währung/Currency/Monnaie Betrag/Amount/Montant
[ ]	Scheck Cheque Chèques	Ausfüllung freigestellt / Optional / facultatif
[ ]	Sonstige Unterlagen (einzeln aufführen) Other documents (specify) Autres documents (préciser)	

B. Beigefügte Dokumente / Accompanying documents / Eléments joints

[ ] Gesonderte unterzeichnete Vollmacht
Separate signed power of attorney
Pouvoir distinct signé

Die genannten Unterlagen sind am oben genannten Tag eingegangen. Die in der Kontrolliste (Feld VIII) des PCT-Antragformulars RO/101 angegebenen Blattzahlen wurden bei Eingang nicht geprüft. Die Anmeldung hat ebenfalls oben angeführte Anmeldenummer erhalten / The said items were received on the date indicated above. No check was made on receipt that the number of sheets indicated in the check list (box VIII) of the PCT Request Form RO/101 were correct. The application has been assigned the above-indicated application number / Les documents mentionnées ont été reçus à la date indiquée. L'exactitude du nombre de feuilles indiqué au bordereau (cadre VIII) du formulaire de requête PCT RO/101 n'a pas été contrôlée lors du dépot. Le numéro figurant ci-dessus a été attribué à la demande de brevet.

Europäisches Patentamt
European Patent Office
Office européen des brevets

Unterschrift / Amtsstempel / Signature / Official stamp (Signature of the person authorized)

A. Keilner

CERAMIDE ANALOGS, PROCESS FOR THEIR PREPARATION AND THEIR USE AS ANTITUMOR AGENTS

Field of the invention

The present invention concerns the ceramide analog compounds of the general formula (I) specified below, their corresponding preparation process, and their use in the preparation of pharmaceutical formulations with an antitumor effect.

State of the art

Ceramides are lipids composed of a fatty acid and sphingosine joined together by an amide link; they are generated by sphingomyelin, a sphingolipid occurring in the membranes of eukaryote cells due to the action of the enzyme sphingomyelinase, or they are synthesized by the action of the enzyme ceramide synthetase.

Sphingolipids such as sphingomyelin have always been considered as stable and metabolically inactive structural components of the membranes. It is only in the last decade that it has been demonstrated, instead, that sphingolipids have an active role in the mechanisms regulating cell response to exogenous stimuli, as well as in regulating cell growth, differentiation, transformation and adhesion.

It has also recently been demonstrated that the products of the demolition of sphingolipids, i.e. ceramides and sphingosine, play an important part in regulating the transmission mechanisms of the signals controlled by the membrane

sphingolipids (Teruyuki Sakai et al., *Exp Opin Ther Patents* [1988] 8 [12]: 1673-1682). In particular, the distinctive characteristic of these products seems to be their involvement in the antiproliferative mechanisms of cell regulation, such as cell growth inhibition, the induction of cell differentiation and programmed cell death, or apoptosis.

Apoptosis has recently been the object of numerous studies (e.g. Ross A. Kinloch et al., *TiPS*, Jan 1999 [20]: 35-42), because this phenomenon lends itself to pharmacological "manipulation": in fact, a reduction in the frequency of the onset of cell apoptosis can have severe pathological consequences and facilitate tumor growth, hence the therapeutic potential of all those compounds that are capable of stimulating apoptosis.

From in-depth studies it has emerged that the ceramides in the cell membranes act as intracellular "effectors" of apoptosis, and therefore as potential inhibitors of tumor growth.

In order to boost this capacity of the endogenous ceramides pharmacologically, the ideal strategy seems to be to develop endogenous ceramide analogs that mimic their effects, are stable in relation to metabolization of the sphingosine ceramide and have an inhibitory effect on the ceramidase in order to prevent the generation of sphingosine, which represents a factor that stimulates proliferation, starting from the endogenous sources of ceramides.

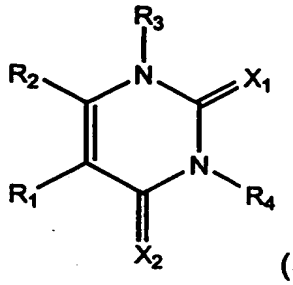
Such ceramide analogs should also have the capacity to penetrate the cell membrane.

There is consequent a need for ceramide analog compounds that are capable of crossing the cell membranes, penetrating inside the cells and mimicking the

various properties of the ceramides, and particularly that of inducing apoptosis in human cancer cells.

Summary of the invention

The Applicant has now surprisingly discovered that the ceramide analog compounds of formula (I):



(I)

wherein:

X_1 and X_2 are selected between O and S;

R_1 and R_2 are selected between $-(CH_2)_{13}CH_3$ and alkyl or alkylene groups with from 2 to 6 carbon atoms, linear or branching, unsubstituted or substituted with one or more substituents selected among aromatic, primary, secondary and tertiary aminic, quaternary ammonium, carboxylic, hydroxylic, polyoxyalkylic and ethereal groups, aminoacids, halogen atoms or saccharidic portions, providing that between R_1 and R_2 only one is always $-(CH_2)_{13}CH_3$,

R_3 and R_4 are selected between H and alkyl or alkylene groups with from 2 to 6 carbon atoms, linear or branching, unsubstituted or substituted with one or more substituents selected among aromatic, primary, secondary and tertiary aminic, quaternary ammonium, carboxylic, hydroxylic, polyoxyalkylic and ethereal groups, aminoacids, halogen atoms or saccharidic portions,

are capable of penetrating inside the biological membranes and effectively inducing apoptosis of the cancer cells.

The compounds of the general formula (I) considered in the present invention have therefore proved suitable for the preparation of pharmaceutical formulations for the treatment of tumors.

The object of the present invention is therefore represented by the compounds of the general formula (I), their corresponding preparation process, and their use in the preparation of pharmaceutical formulations for use in the treatment of tumors.

The characteristics and advantages of the compounds of the general formula (I) according to the present invention will be illustrated in detail in the following description.

DETAILED DESCRIPTION OF THE INVENTION

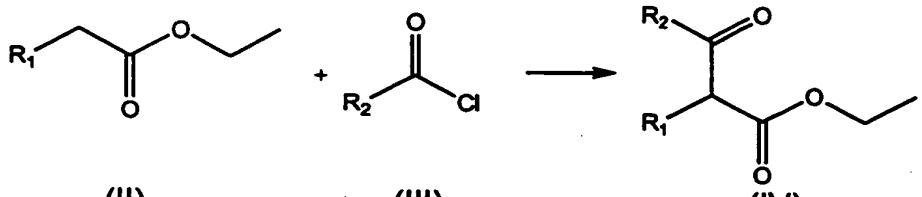
The present invention refers to the compounds of the general formula (I), as defined above. Said compounds (I) have proved capable of penetrating inside the biological membranes and effectively inducing the apoptosis of cancer cells. The following compounds have proved particularly effective and highly cytotoxic:

- compound of formula (I) where X_1= S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = ethyl, and R_3 = R_4 = H [compound **(3)**];
- compound of formula (I) where X_1= X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = ethyl, and R_3 = R_4 = H [compound **(4)**];
- compound of formula (I) where X_1= X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H [compound **(6)**];
- compound of formula (I) where X_1= X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and R_3 = R_4 = H [compound **(10)**];

The present compounds of formula (I) can be conveniently prepared by processes well known in the art. For example, a process for the preparation of the present

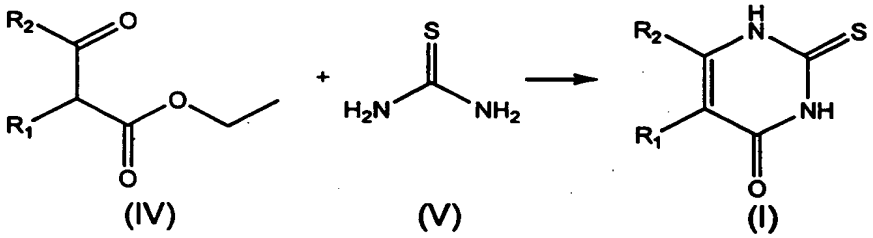
compounds of formula (I) wherein $R_3 = R_4 = H$ includes the following steps:

i) reaction of the ethyl ester (II) with acid chloride (III) to obtain the ß-ketoester of formula (IV):



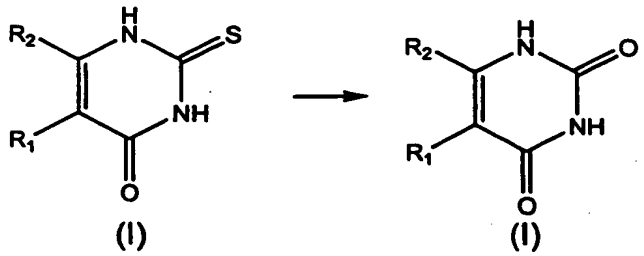
(II) (III) (IV)

ii) reaction of the ß-ketoester of formula (IV) with thiourea (V) to obtain the compound of formula (I) where $X_1 = S$, $X_2 = O$:



(IV) (V) (I)

iii) reaction compound of formula (I) where $X_1 = S$, $X_2 = O$, with refluxed chloroacetic acid to obtain the compound of formula (I) wherein $X_1 = X_2 = O$:



(I) (I)

wherein X_1, X_2, R_1 and R_2 have above-specified meanings.

Step i) of the said process is generally carried out in an organic solvent, such as THF, at a temperature of 0°C. Said reaction is preferably carried out in an inert gas atmosphere.

The reaction product of formula (IV) can be recovered from the reaction mixture by addition of a saturated NH_4Cl solution and subsequent extraction with diethyl ether.

Step ii) of the present process is carried out by means of the addition of thiourea in ethanol and sodium ethoxide on the raw reaction product coming from step i), without the need for any purification. In step ii) temperature is preferably maintained around 90°C. The reaction product is generally recovered from the reaction mixture by acidification at pH 2, e.g. by adding conc. HCl, and filtration of the resulting precipitate, which can be purified, if necessary, by washing with acetone.

The reaction product obtained in step ii) can be further purified by chromatography on silica gel, preferably using a mixture of ethyl acetate and petroleum ether in proportions of 2:1 as an eluant.

Step iii) of the process according to the above procedure is generally carried out by adding chloroacetic acid to the product coming from step ii), e.g. in the form of a 10% aqueous solution, and reflux heated. The crude residue thus obtained can then be purified by washing with absolute ethanol and then with diethyl ether.

The product coming from step iii) can be further purified by chromatography on silica gel, preferably using a mixture of ethyl acetate and hexane in proportions of 1:2 as an eluant.

The present compounds of formula (I) wherein R_3 and/or R_4 are different from H, can be prepared from the ß-ketoester of formula (IV) or from the compounds of formula (I) wherein $R_3 = R_4 = H$, obtained for example as explained above, by means of well-known processes.

Other processes for the preparation of the present formula (I) compounds are disclosed in the examples.

The compounds of formula (I) according to the present invention can be formulated with pharmaceutically acceptable excipients and/or diluents in order to prepare pharmaceutical formulations suitable for the treatment of tumor pathologies.

The following examples are given as a partial illustration of the present invention.

EXAMPLE 1

Preparation of the compound of formula (I) where X_1 = S, X_2 = O, R_2 = –$(CH_2)_{13}CH_3$, R_1 = ethyl, and R_3 = R_4 = H [compound (1)]

A solution prepared by dissolving 0.37 g of ethyl butyrate in 2 ml of anhydrous tetrahydrofuran (THF) is added drop by drop, at a temperature of 0°C and in an argon gas atmosphere, to 1.9 ml of a 2M solution of lithiodiisopropylamine (LDA) in anhydrous THF. After 30 minutes of agitation at 0°C, the reaction mixture is added to a solution obtained by dissolving 1 g of pentadecanol chloride (3.8 mmol) in 5 ml of anhydrous THF, previously cooled to 0°C. The resulting mixture is constantly agitated at room temperature for 12 hours, then added to a saturated solution of NH_4Cl. The organic phase is separated from the aqueous phase, then extracted with diethyl ether. The organic extracts are combined, washed with a saturated aqueous solution of NaCl, dried with anhydrous Na_2SO_4 and then evaporated until dry to provide a crude residue (1.20 g) composed almost exclusively of ß-ketoester (IV) where R_2 = –$(CH_2)_{13}CH_3$ and R_1 = ethyl. [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.83-0.94 (m, 6H), 1.07 (t, 3H, *J* = 7.4 Hz), 1.15-1.36 (m, 24H), 1.81-2.02 (m, 2H), 2.11-2.57 (m, 2H), 3.34 (t, 1H, *J* = 7.3 Hz), 4.15 (q, 2H, *J* = 7.3 Hz). MS m/e 340 M^+].

The resulting crude residue (1.20 g) containing the ß-ketoester (IV) where R_2 = –

$(CH_2)_{13}CH_3$ and R_1 = ethyl, is dissolved in 20 ml of absolute ethanol and then added to 3.61 g of thiourea (47.5 mmol) and 6.47 g of sodium ethoxide (95.1 mmol). The mixture is agitated for 60 minutes at 90°C. After cooling to room temperature, the reaction mixture is filtered and the filtrate is evaporated until dry; the residue thus obtained is then restored with a mixture of water and THF in proportions of 10:1 until it has become completely soluble. The solution, cooled to 0°C, is acidified to pH 2 with conc. HCl; the precipitate that develops is filtered and washed with small quantities of acetone and provides a crude residue that is purified by chromatography on silica gel using ethyl acetate and petroleum ether in proportions of 2:1 as an eluant, finally obtaining 290 mg (0.82 mmol; yield = 26%) of the required compound of formula (I) (m.p. = 167-169°C; [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.89 (t, 3H, *J* = 6.2 Hz), 1.09 (t, 3H, *J* = 7.4 Hz), 1.17-1.36 (m, 24H), 2.34-2.49 (m, 4H), 8.88 (br, 1H, D_2O exchangeable), 9.81 (br, 1H, D_2O exchangeable); MS m/e 352 M^+).

EXAMPLE 2

Preparation of the compound of formula (I) where X_1 = X_2 = O, R_2 = $-(CH_2)_{13}CH_3$, R_1 = ethyl, and R_3 = R_4 = H [compound (2)]

160 mg (0.45 mmol) of the product (1) obtained as described in Example 1 are added to 11.4 ml of a 10% aqueous solution of chloroacetic acid and the mixture thus obtained is reflux heated for 12 hours. The resulting precipitate is then filtered, washed first with absolute ethanol, then with diethyl ether, to obtain a crude residue that, after purification by chromatography on silica gel using a mixture of ethyl acetate and hexane in proportions of 1:2 as an eluant, gave rise to 48 mg (0.14 mmol, yield = 32%) of the required pure compound (m.p. = 132-

134°C; [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.2 Hz), 1.06 (t, 3H, *J* = 7.4 Hz), 1.15-1.36 (m, 24H), 2.31-2.49 (m, 4H), 9.06 (br, 1H, D_2O exchangeable), 9.89 (br, 1H, D_2O exchangeable); MS m/e 336 M^+).

EXAMPLE 3

Preparation of the compound of formula (I) wherein X_1= S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = ethyl, and R_3 = R_4 = H [compound (3)]

A solution obtained by dissolving 1 g of ethyl palmitate (3.52 mmol) in 3 ml of anhydrous THF is added drop by drop, at a temperature of 0°C in an argon gas atmosphere, to 2.1 ml of a 2M solution of lithiodiisopropylamine (LDA) in anhydrous THF. After 30 minutes of agitation at 0°C, the reaction mixture is added to a solution obtained by dissolving 2.39 g (4.23 mmol) of propionyl chloride in 5 ml of anhydrous THF. The resulting mixture is constantly agitated at room temperature for 12 hours, then added to a saturated solution of NH_4Cl. The organic phase is separated from the aqueous phase, then extracted with diethyl ether. The organic extracts are combined, washed with a saturated aqueous solution of NaCl, dried with anhydrous Na_2SO_4 and then evaporated until dry to provide a crude residue (1.31 g) composed almost exclusively of ß-ketoester (IV) where R_1 = –$(CH_2)_{13}CH_3$ and R_2 = ethyl. [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.79-0.92 (m, 6H), 1.11 (t, 3H, *J* = 7.6 Hz), 1.17-1.39 (m, 24H), 1.48-1.62 (m, 2H), 2.26 (q, 2H, *J* = 7.6 Hz), 3.36 (t, 1H, *J* = 7.3 Hz), 4.15 (q, 2H, *J* = 7.2 Hz); MS m/e 340 M^+].

1.31 g of the resulting crude residue containing the ß-ketoester (IV) where R_1 = –$(CH_2)_{13}CH_3$ and R_2 = ethyl, is dissolved in 20 ml of absolute ethanol and then added to 4.01 g of thiourea (52.8 mmol) and 7.18 g of sodium ethoxide (105.6 mmol). The mixture is agitated for 60 minutes at 90°C. After cooling to room

temperature, the reaction mixture is filtered and the filtrate is evaporated until dry; the residue thus obtained is then treated with a mixture of water and THF in proportions of 10:1 until it has become completely soluble. The solution is cooled to 0°C and acidified to pH 2 with conc. HCl; the precipitate that develops due to acidification is filtered and washed with small quantities of acetone and provides a crude residue that is purified by chromatography on silica gel using ethyl acetate and petroleum ether in proportions of 2:1 as an eluant, finally obtaining 310 mg (0.88 mmol; yield = 25%) of a product that coincides with the required pure compound **3** (m.p. = 100-102°C; [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.01 (t, 3H, *J* = 7.4 Hz), 1.18-1.38 (m, 24H), 2.35 (t, 2H, *J* = 7.4 Hz), 2.48 (q, 2H, *J* = 7.6 Hz), 9.08 (br, 1H, D_2O exchangeable), 9.73 (br, 1H, D_2O exchangeable); MS m/e 352 M^+).

EXAMPLE 4

Preparation of the compound of formula (I) wherein $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ [compound (4)]

160 mg (0.45 mmol) of the compound (**3**) obtained as described in Example 3 are added to 11.4 ml of a 10% aqueous solution of chloroacetic acid and the mixture thus obtained is reflux heated for 12 hours. The resulting precipitate is then filtered, washed first with absolute ethanol, then with diethyl ether, to obtain a crude residue that, after purification by chromatography on silica gel using a mixture of ethyl acetate and hexane in proportions of 1:2 as an eluant, gave rise to 57 mg (0.17 mmol, yield = 38%) of the compound (**4**) (m.p. = 110-112°C; [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.89 (t, 3H, *J* = 6.4 Hz), 1.02 (t, 3H, *J* = 7.4 Hz), 1.12-1.42 (m, 24H), 2.34 (t, 2H, *J* = 7.2 Hz), 2.49 (q, 2H, *J* = 7.6 Hz), 9.15 (br, 1H, D_2O

exchangeable), 9.53 (br, 1H, D_2O exchangeable); MS m/e 336 M^+).

EXAMPLE 5

Preparation of the compound of formula (I) wherein X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H [compound (5)]

Compound (5) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 366 M^+.

EXAMPLE 6

Preparation of the compound of formula (I) wherein X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H [compound (6)]

Compound (6) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 350 M^+.

EXAMPLE 7

Preparation of the compound of formula (I) wherein X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-butyl, and R_3 = R_4 = H [compound (7)]

Compound (7) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 380 M^+.

EXAMPLE 8

Preparation of the compound of formula (I) wherein X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-butyl, and R_3 = R_4 = H [compound (8)]

Compound (8) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 364 M^+.

EXAMPLE 9

Preparation of the compound of formula (I) wherein X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and R_3 = R_4 = H [compound (9)]

Compound (9) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 380 M^+.

EXAMPLE 10

Preparation of the compound of formula (I) wherein $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 =i-butyl, and $R_3 = R_4 = H$ [compound (10)]

Compound (10) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 364 M^+.

EXAMPLE 11

Preparation of the compound of formula (I) wherein $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = neopentyl, and $R_3 = R_4 = H$ [compound (11)]

Compound (11) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 394 M^+.

EXAMPLE 12

Preparation of the compound of formula (I) wherein $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = neopentyl, and $R_3 = R_4 = H$ [compound (12)]

Compound (12) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 378 M^+.

EXAMPLE 13

Preparation of the compound of formula (I) wherein $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = 2-phenyl-ethyl, and $R_3 = R_4 = H$ [compound (13)]

Compound (13) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 428 M^+.

EXAMPLE 14

Preparation of the compound of formula (I) wherein $X_1 = X_2 = O$, $R_1 = -$

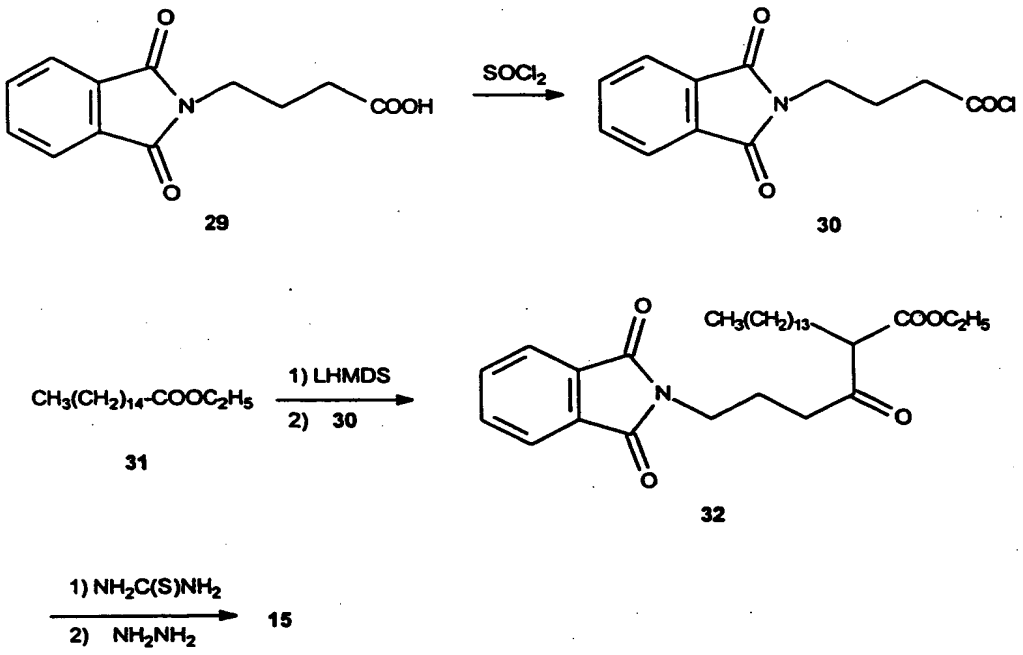
$(CH_2)_{13}CH_3$, R_2 = 2-phenyl-ethyl, and $R_3 = R_4$ = H [compound (14)]

Compound (**14**) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 412 M^+.

EXAMPLE 15

Preparation of the compound of formula (I) wherein X_1 =S, X_2 = O, R_1 = – $(CH_2)_{13}CH_3$, R_2 = -$(CH_2)_3NH_2$ and $R_3 = R_4$ = H [compound (15)]

Scheme 1



Compound (**15**) was prepared following the procedure described in the above Scheme 1.

Synthesis of β-ketoester (**32**). 2.4 g (10 mmol) of 4-phthalimidoburyric acid (**29**) (prepared as described in G. Talbot, R. Gaudry, L. Berlinguet *Can. J. Chem.* **1958**, *36*, 593-596) was dissolved in 7.5 ml of $SOCl_2$ and the mixture was refluxed under nitrogen for 3 hours. Excess of $SOCl_2$ was then removed under a nitrogen flow and the resulting acid chloride (**30**) was used in the next step without further

purification. Separately, a solution of ethyl palmitate (**31**) (1.47 g, 5.16 mmol) in anhydrous THF (6.5 ml) was slowly added to a 1.0 M solution of lithium bis(trimethylsilyl)amide (LHMDS) in THF (6.2 ml, 6.2 mmol) cooled at -20 °C and the resulting mixture was stirred for additional 20 minutes. Acid chloride (**30**) previously prepared as described above, was dissolved in anhydrous THF (10 ml), cooled at -20 °C, and added via cannula to the solution containing (**31**) and LHMDS at the same temperature. The mixture was stirred at -20 °C for 30 minutes and then at room temperature for 2 hours. The reaction was quenched with a saturated aqueous solution of ammonium chloride and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-ethyl acetate (8:2) as the eluant, to obtain 0.95 g (1.9 mmol, 37% yield) of pure β-ketoester (**32**) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.4 Hz), 1.23 (t, 3H, *J* = 7.3 Hz), 1.24 (bs, 24H), 2.05 (quintet, 2H, *J* = 7.2 Hz), 2.24-2.34 (m, 4H), 2.51 (t, 2H, *J* = 7.6 Hz), 3.78 (t, 1H, *J* = 6.9 Hz), 4.12 (q, 2H, *J* = 7.1 Hz), 7.69-7.73 (m, 2H), 7.82-7.87 (m, 2H); MS (FAB$^+$) m/z 500 $(M+H)^+$.

Synthesis of thiouracil (**15**). β-Ketoester (**32**) (0.12 g, 0.24 mmol) was dissolved in 2 ml of absolute ethanol. Thiourea (0.024 g, 0.33 mmol) and potassium *t*-butoxyde (0.028 g, 0.25 mmol) were added and the resulting mixture was refluxed for 5 hours. The mixture was then cooled to room temperature and the solvent was removed under vacuum. The residue was treated with 20 ml of water and neutralized with an aqueous solution of acetic acid 0.5 N. The product was extracted with ethyl acetate and the organic layer was washed with brine, dried

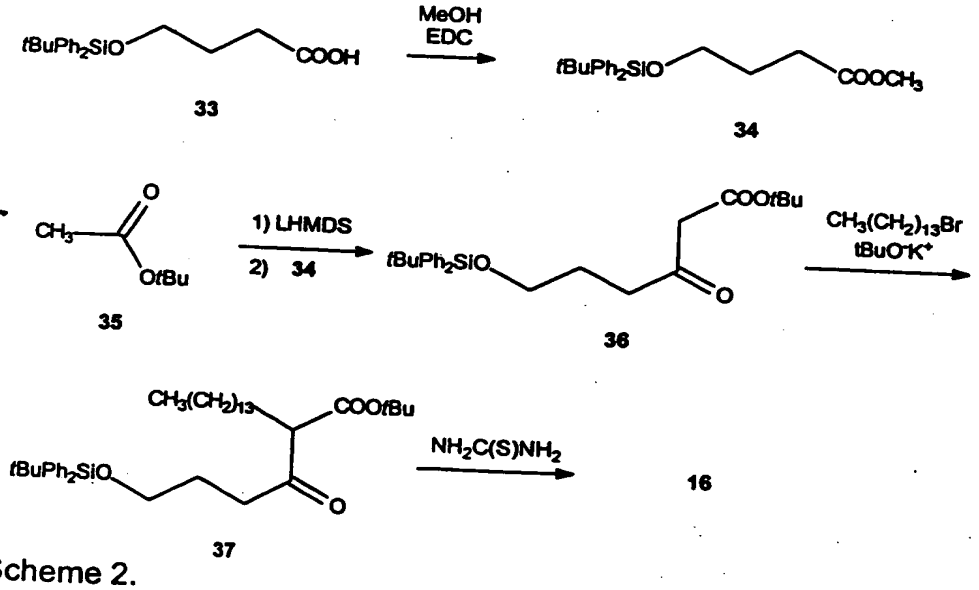
over anhydrous sodium sulfate and concentrated under vacuum. The crude residue was then redissolved in 3 ml of ethanol, treated with 0.06 ml of hydrazine monohydrate (1.3 mmol), and the mixture was refluxed overnight. The resulting suspension was cooled to room temperature. The white solid was collected by filtration, washed with small portions of ethyl acetate, and dried under vacuum, to give 51 mg (0.13 mmol, 54% yield) of product (**15**): m.p. 123-125 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, 3H, J = 6.4 Hz), 1.26 (bs, 24H), 1.77 (m, 2H), 2.29-2.45 (m, 6H), 8.87 (bs, 1H), 9.19 (bs, 1H); MS (FAB$^+$) m/z 381 $(M+H)^+$.

EXAMPLE 16

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OSiPh_2t$-Bu and R_3 = R_4 = H [compound (16)]

Compound (**16**) was prepared following the procedure described in the following

Scheme 2



Scheme 2.

Synthesis of methyl ester (**34**). A solution of acid (**33**) (1.15 g, 3.36 mmol) (prepared as in: A.G.M. Barrett, J.A. Flygare *J. Org. Chem.* **1991**, *56*, 638-642) in methanol (25 ml) was treated with 1.62 g (8.44 mmol) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC). The resulting solution was stirred under nitrogen at room temperature for 3.5 hours. The solvent is then removed under vacuum and the residue was diluted with chloroform (100 ml) and water (50 ml). The organic layer was washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-ethyl acetate (9:1) as the eluant, to obtain 0.59 g (1.6 mmol, 49% yield) of pure ester (**34**) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.05 (s, 9H), 1.88 (tt, 2H, *J* = 7.7, 5.9 Hz), 2.47 (t, 2H, *J* = 7.5 Hz), 3.66 (s, 3H), 3.68 (t, 2H, *J* = 6.0 Hz), 7.37-7.42 (m, 6H), 7.63-7.68 (m, 4H).

Synthesis of β-ketoester (**36**). A solution of *t*-butyl acetate (**35**) (4.24 g, 36.5 mmol) in anhydrous THF (40 ml) previously cooled at -78 °C was added drop by drop via cannula under argon to a 1M solution of LHMDS in THF (51.5 ml, 51.5 mmol). To the resulting solution, previously stirred at the same temperature for 30 minutes, was added drop by drop via cannula another solution of methyl ester (**34**) (4.07 g, 11.4 mmol) in anhydrous THF (20 ml) at -78 °C. The reaction mixture was stirred under argon for 20 minutes at the same temperature, and then 3 more hours at room temperature. The reaction was quenched with 400 ml of saturated aqueous solution of ammonium chloride and extracted with diethyl ether (2 x 300 ml). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-diethyl ether (8:2) as the eluant, to obtain 2.8 g (6.4 mmol, 56%

yield) of pure β-ketoester (**36**) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.04 (s, 9H), 1.46 (s, 9H), 1.84 (quintet, 2H, *J* = 6.7 Hz), 2.66 (t, 2H, *J* = 7.3 Hz), 3.34 (s, 2H), 3.67 (t, 2H, *J* = 6.0 Hz), 7.37-7.43 (m, 6H), 7.62-7.67 (m, 4H); MS (FAB^+) m/z 441 $(M+H)^+$, 385 $(M+H\text{-isobutene})^+$.

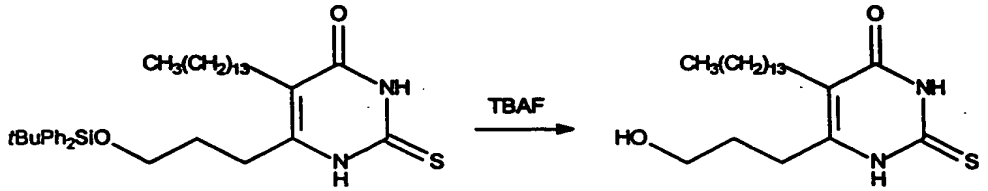
Synthesis of alkylated β-ketoester (**37**). A solution of β-ketoester (**36**) (2.79 g, 6.34 mmol) in anhydrous 1,2-dimethoxyethane (DME) (17 ml) was added to a solution of potassium *tert*-butoxide (0.85 g, 6.97 mmol) in anhydrous DME (7 ml). The resulting solution was stirred at room temperature for 20 minutes, after which time 1.7 ml (1.6 g, 5.7 mmol) of 1-bromotetradecane were added. The reaction mixture was stirred at 80 °C for 2 hours. The reaction was quenched with 150 ml of a saturated aqueous solution of ammonium chloride and extracted with diethylether (2 x 300 ml). The organic layer was washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-diethyl ether (9:1) as the eluant, to obtain 1.16 g (1.82 mmol, 32% yield) of pure mono-alkylated product (**37**) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, 3H, *J* = 6.2 Hz), 1.04 (s, 9H), 1.25 (bs, 24H), 1.43 (s, 9H), 1.76-1.89 (m, 4H), 2.64 (td, 2H, *J* = 7.3, 4.4 Hz), 3.13 (t, 1H, *J* = 7.3 Hz), 3.66 (t, 2H, *J* = 6.0 Hz), 7.34-7.43 (m, 6H), 7.62-7.67 (m, 4H); MS (FAB^+) m/z 581 $(M+H\text{-isobutene})^+$, 563 $(M\text{-}t\text{BuO})^+$.

Synthesis of thiouracil (**16**). A solution containing alkylated β-ketoester (**37**) (1.16 g, 1.82 mmol) in absolute ethanol (24 ml) in a screw-cap sealed vial was treated first with 0.19 g (2.6 mmol) of thiourea and then with 0.25 g (2.0 mmol) of potassium *tert*-butoxide. The resulting solution was stirred at 100 °C for 6 hours. The solvent was then removed under vacuum. The residue was diluted with water

and neutralized to pH = 6-7 with 0.5 N acetic acid. The product was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-diethyl ether (8:2) as the eluant, to obtain 0.52 g (0.84 mmol, 46% yield) of pure thiouracil product (**16**) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.2 Hz), 1.10 (s, 9H), 1.24 (bs, 24 H), 1.74 (quintet, 2H, *J* = 6.8 Hz), 2.34 (t, 2H, *J* = 7.4 Hz), 2.60 (t, 2H, *J* = 7.5 Hz), 3.74 (t, 2H, *J* = 5.8 Hz), 7.40-7.46 (m, 6H), 7.66-7.70 (m, 4H), 9.29 (bs, 1H), 9.55 (bs, 1H); MS (FAB$^+$) m/z 547 $(M+H-NHC(S)NH)^+$.

EXAMPLE 17

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OH$ and R_3 = R_4 = H [compound (17)]



16 **17**

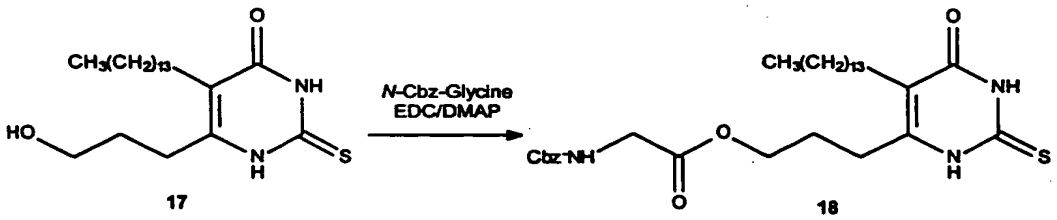
Scheme 3

According to the above Scheme 3 the silyl ether **16** (0.16 g, 0.26 mmol) was treated with 0.8 ml of a 1M solution of tetrabutylammonium fluoride (TBAF) in THF (0.8 mmol) under argon at room temperature for 2 hours. The solvent was then removed under vacuum and the residue was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-ethyl acetate (1:1) as the eluant, to obtain 0.079 g (0.21 mmol, 81% yield) of pure deprotected alcohol (**17**) as a white solid: m.p. 128-

130 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, 3H, *J* = 6.7 Hz), 1.25 (bs, 24H), 1.91 (quintet, 2H, *J* = 6.1 Hz), 2.37 (pseudo t, 2H, *J* = 7.4 Hz), 2.67 (pseudo t, 2H, *J* = 6.3 Hz), 3.84 (t, 2H, *J* = 5.6 Hz), 9.16 (bs, 1H), 10.52 (bs, 1H); MS (EI, 70 eV) m/z 382 $(M)^+$, 365 $(M\text{-}OH)^+$, 323 $(M\text{-}NHC{=}S)^+$.

EXAMPLE 18

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = -$(CH_2)_3OC(O)CH_2NH$-Cbz and R_3 = R_4 = H [compound (18)]



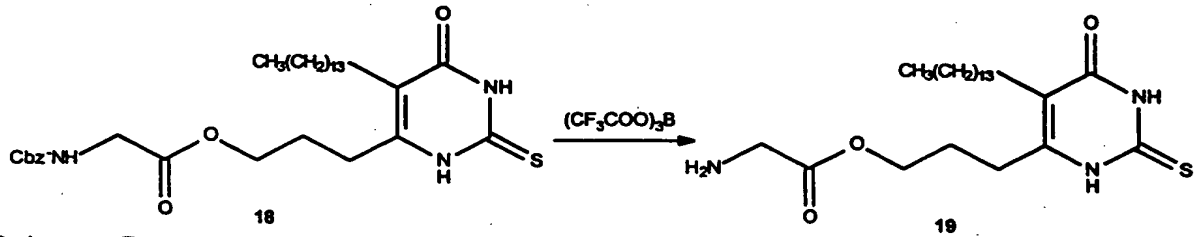
Scheme 4

According to scheme 4 a solution of the alcohol (**17**) (0.038 g, 0.099 mmol) in anhydrous THF (2.5 ml) was sequentially treated with 0.031 g (0.15 mmol) of *N*-carbobenzyloxyglycine (*N*-Cbz-Gly), 0.034 g (0.18 mmol) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), and 0.0012 g (0.0096 mmol) of 4-(dimethylamino)pyridine (DMAP). The mixture was stirred at room temperature for 5 hours under argon. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography using hexane-ethyl acetate (1:1) as the eluant, to obtain 0.052 g (0.091 mmol, 92% yield) of product (**18**) as a thick syrup: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, J = 6.6 Hz), 1.25 (bs, 24H), 1.91 (m, 2H), 2.31 (t, 2H, J = 7.7 Hz), 2.47 (t, 2H, J = 7.7 Hz), 4.07 (d, 2H, J = 5.9 Hz), 4.27 (t, 2H, J = 5.2 Hz), 5.24 (s, 2H), 5.52 (t, 1H, J = 5.8 Hz), 7.31-7.38 (m, 5H), 10.09 (bs, 1H), 10.85 (bs 1H); MS (FAB^+) m/z 574

$(M+H)^+$, 532 $(M-C(S)+H)^+$.

EXAMPLE 19

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OC(O)CH_2NH_2$ and R_3 = R_4 = H [compound (19)]

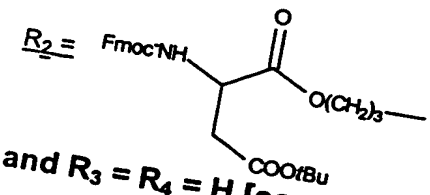


Scheme 5

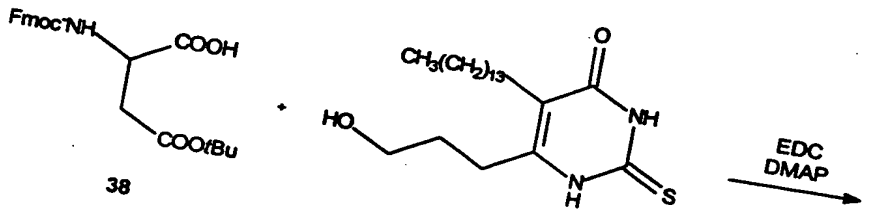
According to the above scheme 5 a solution of Cbz-protected compound (**18**) (0.032 g, 0.055 mmol) in trifluoroacetic acid (1 ml) was treated with 0.22 mmol of freshly prepared boron tris(trifluoroacetate) (prepared as reported in: J. Pless, W. Bauer *Angew. Chem. Int. Ed.* **1973**, *12*, 147-148) at 0 °C under argon. The mixture was stirred for 1 hour at the same temperature and overnight at room temperature. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography using a mixture dichloromethane : acetone 7 : 3 as the eluant, to obtain 0.020 g (0.045 mmol, 82% yield) of product (**19**) as a thick syrup: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.4 Hz), 1.25 (bs, 24H), 1.72 (m, 2H), 2.32 (m, 2H), 2.63 (m, 2H), 3.61 (t, 2H, *J* = 7.0 Hz), 4.30 (t, 2H, *J* = 6.6 Hz); MS (FAB$^+$) m/z 365 $(M-NHC(S)NH)^+$.

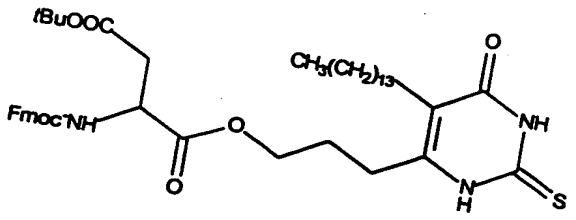
EXAMPLE 20

Preparation of the compound of formula (I) where X_1=S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$,



and R_3 = R_4 = H [compound (20)]





e 6

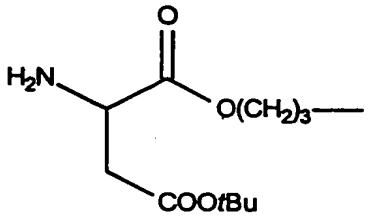
ng to the above scheme 6 a solution of alcohol (17) (0.120 g, 0.314 mmol) ydrous THF (10 ml) was treated sequentially with *N*-(9-methoxycarbonyl)-L-aspartic acid *tert*-butyl ester (38) (0.194 g, 0.471 -[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) .562 mmol) and 4-(dimethylamino)pyridine (DMAP) (0.0077 g, 0.063 mixture was stirred under argon at room temperature for 5 hours. The removed under vacuum and the residue was purified by silica gel atography (hexane - ethyl acetate 1:1) to afford 0.24 g (0.31 mmol, oduct (20) as a syrup: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* (bs, 24H), 1.46 (s, 9H), 1.93 (m, 2H), 2.30 (m, 2H), 2.49 (m, 2H),

2.80 (dd, 1H, *J* = 16.6, 4.8 Hz), 2.93 (dd, 1H, *J* = 16.6, 5.0 Hz), 4.24-4.33 (m, 2H), 4.48-4.54 (m, 2H), 4.67-4.72 (m, 1H), 5.97 (d, 1H), 7.29-7.43 (m, 5H), 7.62 (d, 2H, *J* = 7.2 Hz), 7.76 (d, 2H, *J* = 7.2 Hz), 9.59 (bs, 1H), 10.58 (bs, 1H).

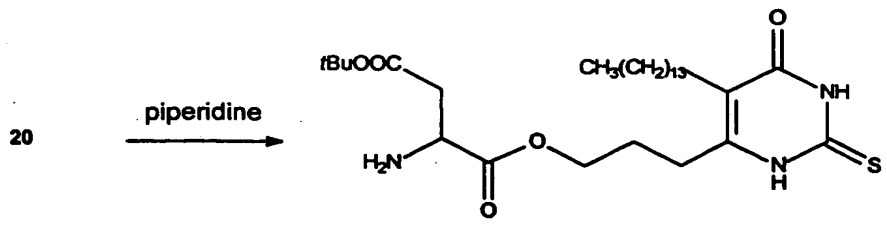
EXAMPLE 21

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$,

R_2 =



and R_3 = R_4 = H [compound (21)]



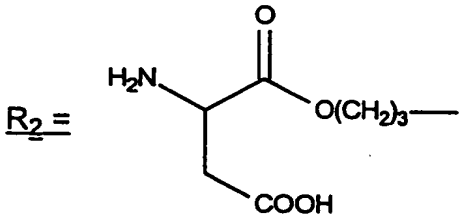
21

Scheme 7

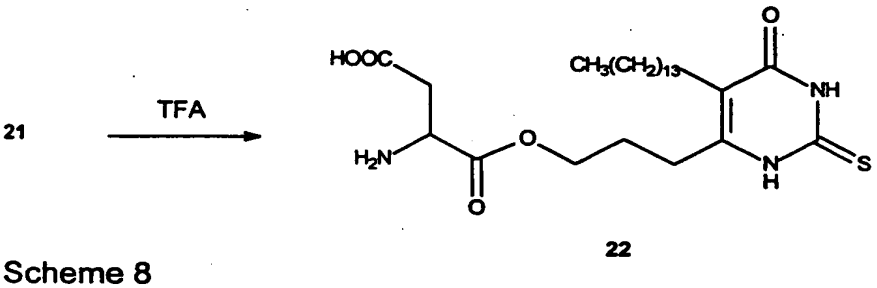
According to the above scheme 7 a solution of Fmoc-protected product (**20**) (0.120 g, 0.155 mmol) in anhydrous dichloromethane (5 ml) was treated with 0.020 g of piperidine (0.23 mmol). The mixture was stirred at room temperature for 30 minutes. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography (hexane - ethyl acetate 3:7) to afford 0.040 g (0.072 mmol, 47 % yield) of product (**20**) as a syrup: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.6 Hz), 1.25 (bs, 24H), 1.46 (s, 9H), 1.94 (m, 2H), 2.33 (t, 2H, *J* = 7.2 Hz), 2.56 (t, 2H, *J* = 7.7 Hz), 2.76 (d, 2H, *J* = 5.9 Hz), 3.94 (t, 1H, *J* = 5.8 Hz), 4.21-4.30 (m, 2H).

EXAMPLE 22

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$,



and R_3 = R_4 = H [compound (22)]



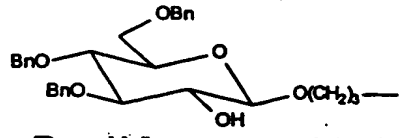
22

Scheme 8

According to the above scheme 8 the *tert*-Butyl ester (**21**) (0.020 g, 0.040 mmol) was treated with 0.2 ml of a 1:1 mixture of trifluoroacetic acid and dichloromethane. The mixture was stirred at room temperature for 1 hour. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography (acetone - methanol, variable ratios from 100:0 to 50:50) to afford 0.012 g (0.021 mmol, 54 % yield) of product (**20**) as a syrup: ^{1}H NMR (CD_3OD, 200 MHz) δ 0.89 (t, 3H, J = 6.8 Hz), 1.29 (bs, 24H), 1.97 (m, 2H), 2.35 (m, 2H), 2.57 (m, 2H), 2.82 (m, 2H), 4.16-4.44 (m, 3H).

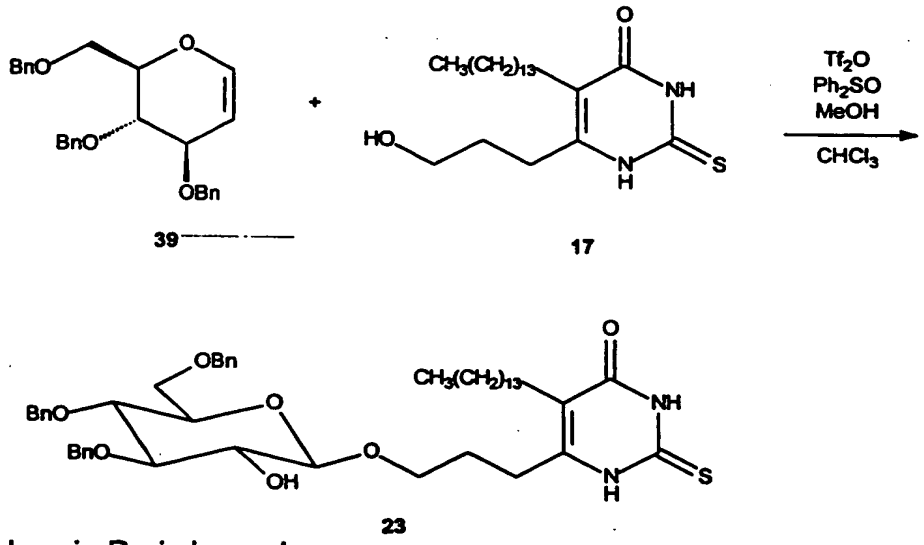
EXAMPLE 23

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$,



and R_3 = R_4 = H [compound (23)]

Scheme 9



wherein Bn is benzyl.

Glucose derivative (**23**) was prepared following a general procedure for direct glycosilation of alcohols with glucal donor (**39**) (as reported in: V. Di Bussolo, Y.-J. Kim, D.Y. Gin *J. Am. Chem. Soc.* **1998**, *120*, 13515-13516), as reported above in scheme 9.

Trifluoromethanesulfonic anhydride (Tf_2O) (0.030 ml, 0.18 mmol) was added to a solution of tri-O-benzil-D-glucal (**39**) (0.050 g, 0.12 mmol), diphenylsulfoxide (0.073 g, 0.36 mmol) and 2,4,6-tri-*t*-butylpyridine (0.104 g, 0.42 mmol) in dry chloroform (5 ml) (distilled over P_2O_5) at - 40 °C. The reaction mixture was stirred at this temperature for 1 hour. Methanol (0.005 ml, 0.12 mmol) and triethylamine

(0.050 ml, 0.36 mmol) were added sequentially at -40 °C and the reaction mixture was stirred at this temperature for 30 minutes, then at 0 °C for 1 hour and at room temperature for 1 hour. A solution of alcohol derivative (17) (0.065 g, 0.17 mmol) in dry chloroform (4 ml) was added at 0 °C, via cannula. Zinc chloride (0.24 ml, 1.0 M in diethyl ether, 0.24 mmol) was added at the same temperature, then the temperature was slowly warmed to room temperature and the reaction mixture stirred at this temperature for 12 hours. The reaction was diluted with chloroform (15 ml) and washed sequentially with saturated aqueous sodium bicarbonate solution (2 x 15 ml) and a saturated aqueous solution of sodium chloride (15 ml). The organic layer was dried (Na_2SO_4) and concentrated, the residue was purified by silica gel column chromatography (hexane-ethyl acetate 6:4) to afford product (23) (0.055 g, 0.067 mmol, 56% yield) as a colourless oil: ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, *J* = 6.3 Hz), 1.25 (bs, 24 H), 1.88 (quintet, 2H, *J* = 6.4 Hz), 2.44 (pseudo *t*, 2H, *J* = 7.5 Hz), 2.65 (t, 2H, *J* = 6.6 Hz), 3.70-3.66 (m, 8H), 4.47 (d, 1H, *J* = 10.6 Hz), 4.52 (d, 1H, *J* = 12.1 Hz), 4.65 (d, 1H, *J* = 12.1 Hz), 4.80 (d, 1H, *J* = 10.8 Hz), 4.86 (d, 1H, *J* = 11.4 Hz), 4.92 (d, 1H, *J* = 11.2 Hz), 5.12 (d, 1H, *J* = 9.2 Hz), 7.09-7.35 (m, 15H), 9.61 (bs, 1H), 11.29 (bs, 1H); MS (FAB$^+$) m/z 815 $(M+H)^+$.

EXAMPLE 24

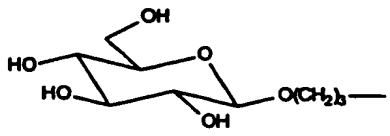
Preparation of the compound of formula (I) where X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = ethyl, R_3 = $-CH_2COOC_2H_5$, and R_4 = H [compound (24)]

Anhydrous $(NH_4)_2SO_4$ (0.0013 g, 0.011 mmol) and 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (0.75 ml, 3.41 mmol) were added, under argon atmosphere, to compound (3) (0.05 g, 0.14 mmol). The resulting suspension was heated at 130 °C and stirred at this temperature for 6 hours. The mixture was then

concentrated at room temperature under a flux of argon. Anhydrous THF (3 ml) was added, and the resulting solution was stirred at - 45°C. Trimethylsilyl triflate (TMS triflate) (0.03 ml, 0.145 mmol) and ethyl bromoacetate (0.046 g, 0.027 mmol) were sequentially added and the mixture was stirred at - 45 °C for 3 hours, then at room temperature for 1 hour. Saturated aqueous $NaHCO_3$ (3 ml) was added and THF was removed under vacuum. The residue was diluted with H_2O (20 ml) and extracted with ethyl acetate (3 x 10 ml). The organic layer was dried with Na_2SO_4 anhydrous, and concentrated to dryness. The residue was purified by semi-preparative thin-layer column chromatography (hexane/ethyl acetate 7:3) to afford product (**24**) (0.010 g, 0.023 mmol, 16% yield) as a colourless oil: ^1H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.6 Hz), 1.17 (t, 3H, *J* = 7.2 Hz), 1.25-1.43 (m, 27H), 2.44 (pseudo t, 2H, *J* = 7.2 Hz), 2.54 (t, 2H, *J* = 7.5 Hz), 3.91 (s, 2H); 4.21 (q, 2H, *J* = 7.3 Hz), 10.88 (bs, 1H);); MS (FAB^+) m/z 439 $(M+H)^+$.

According to procedures analogues to those above reported, the following compounds of formula (I) were prepared:

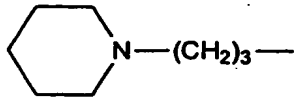
- compound (I) wherein X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$,

R_2 = 

and R_3 = R_4 = H [compound (**25**)];

- compound (I) wherein X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3Br$ and R_3 = R_4 = H [compound (**26**)];

- compound (I) wherein X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$,

R_2 = 

and $R_3 = R_4 = H$ [compound (**27**)];

- compound (I) wherein X_1 =S, X_2 = O, $R_1 = -(CH_2)_{13}CH_3$, $R_2 = -(CH_2)_3N(C_2H_5)_3^+Br^-$ and $R_3 = R_4 = H$ [compound (**28**)].

CYTOTOXICITY TEST

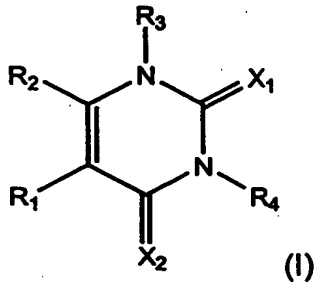
The cytotoxicity of the compounds synthesized 1 - 28 was assessed using a human leukemia cell line called CCRF/CEM. The CCRF/CEM cells were cultured in a culture medium containing RPMI 1640 (90%), bovine fetal sera (10%) and interleukin-2 (100 U/ml). The cytotoxicity assay was performed on 104 CCRF/CEM cells seeded in 35 mm wells in 2 ml of culture medium. The cells were treated with the compounds under consideration for 72 hours and at the end of the period of exposure their number was counted and compared with that of control cells treated with C_2-ceramide in order to establish the percentage of growth inhibition. The concentration capable of inhibiting 50% of cell growth was calculated by non-linear regression of the experimental data as described in M. Macchia, N. Jannitti, G.B. Gervasi, R. Danesi, *J Med Chem*, (1996) 39 (7): 1352-1356.

The resulting values of IC_{50} expressed in μM are given in the following table:

Compound	IC_{50} (μM)
controls	31.6
(3)	1.7
(4)	6.3
(6)	0.97
(9)	13.2
(10)	8.7
(11)	20
(12)	29.1
(13)	20.7
(14)	15.6

Claims

1. Compounds of general formula (I)



(I)

where

X_1 and X_2 are selected between O and S;

R_1 and R_2 are selected between $-(CH_2)_{13}CH_3$ and alkyl or alkylene groups with from 2 to 6 carbon atoms, linear or branching, unsubstituted or substituted with one or more substituents selected among aromatic, primary, secondary and tertiary aminic, quaternary ammonium, carboxylic, hydroxylic, polyoxyalkyl and ethereal groups, aminoacids, halogen atoms or saccharidic portions, providing that between R_1 and R_2 only one is always $-(CH_2)_{13}CH_3$,

R_3 and R_4 are selected between H and alkyl or alkylene groups with from 2 to 6 carbon atoms, linear or branching, unsubstituted or substituted with one or more substituents selected among aromatic, primary, secondary and tertiary aminic, quaternary ammonium, carboxylic, hydroxylic, polyoxyalkyl and ethereal groups, aminoacids, halogen atoms or saccharidic portions.

2. The compounds of general formula (I) according to claim 1, where:

$X_1 = S$, $X_2 = O$, R_1 = ethyl, $R_2 = -(CH_2)_{13}CH_3$, and $R_3 = R_4 = H$ (compound **1**);

$X_1 = X_2 = O$, R_1 = ethyl, $R_2 = -(CH_2)_{13}CH_3$, and $R_3 = R_4 = H$ (compound **2**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ (compound **3**);

$X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ (compound **4**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H (compound **5**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H (compound **6**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-butyl, and R_3 = R_4 = H (compound **7**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-butyl, and R_3 = R_4 = H (compound **8**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and R_3 = R_4 = H (compound **9**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and R_3 = R_4 = H (compound **10**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = neopentyl, and R_3 = R_4 = H (compound **11**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = neopentyl, and R_3 = R_4 = H (compound **12**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = 2-phenyl-ethyl, and R_3 = R_4 = H (compound **13**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = 2-phenyl-ethyl, and R_3 = R_4 = H (compound **14**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2= $-(CH_2)_3NH_2$, and R_3 = R_4 = H (compound **15**);

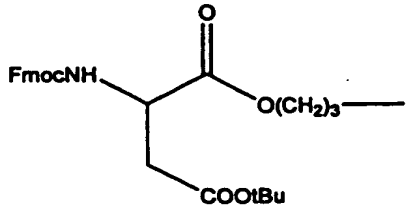
X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OSiPh_2t$-Bu, and R_3 = R_4 = H (compound **16**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OH$, and R_3 = R_4 = H (compound **17**);

X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OC(O)CH_2NH$-Cbz, and R_3 = R_4 = H (compound **18**);

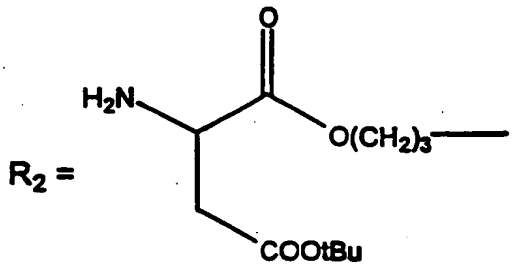
X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OC(O)CH_2NH_2$, and R_3 = R_4 = H (compound **19**);

X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 =



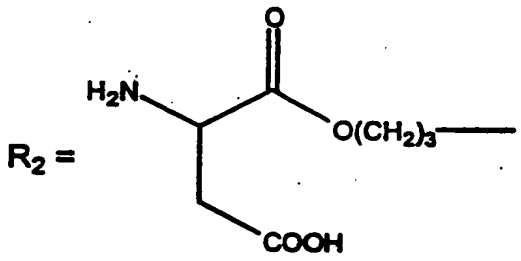
and $R_3 = R_4 = H$ (compound **20**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



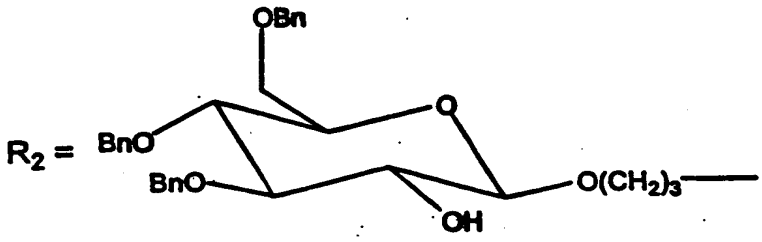
and $R_3 = R_4 = H$ (compound **21**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



and $R_3 = R_4 = H$ (compound **22**);

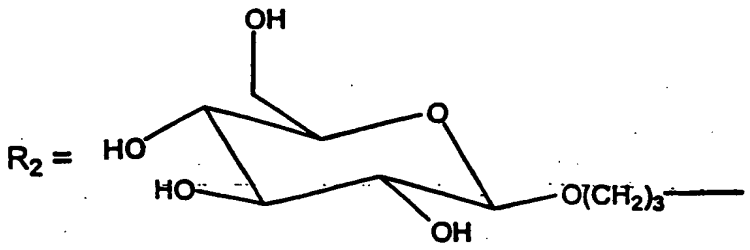
$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



wherein Bn is benzyl and $R_3 = R_4 = H$ (compound **23**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, $R_3 = -CH_2COOC_2H_5$, and $R_4 = H$ (compound **24**);

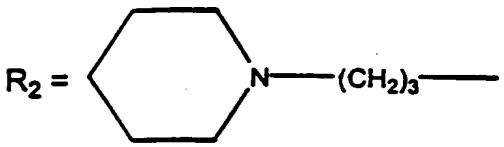
$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



and $R_3 = R_4 = H$ (compound **25**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, $R_2 = -(CH_2)_3Br$, and $R_3 = R_4 = H$ (compound **26**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



and $R_3 = R_4 = H$ (compound **27**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, $R_2 = -(CH_2)_3N(C_2H_5)_3^+Br^-$, and $R_3 = R_4 = H$ (compound **28**).

3. The compounds of general formula (I) according to claim 1, where:

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ (compound **3**);

$X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ (compound **4**);

$X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and $R_3 = R_4 = H$ (compound **6**);

$X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and $R_3 = R_4 = H$ (compound **10**).

4. Pharmaceutical preparations including as their active ingredient at least one of the compounds of the general formula (I) described in claims 1-3, and/or their pharmaceutically acceptable derivatives or salts, together with excipients and/or

diluents.

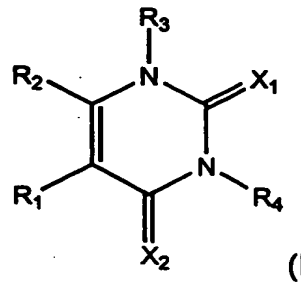
5. Use of the compounds of the general formula (I) described in claims 1-3 for the preparation of pharmaceutical formulations.

6. The use according to claim 5, for the preparation of pharmaceutical formulations for use in the treatment of tumours.

CERAMIDE ANALOGS, PROCESS FOR THEIR PREPARATION AND THEIR USE AS ANTITUMOR AGENTS

Abstract

The present invention is directed to ceramide analog compounds of general formula (I)



(I)

the process for their preparation and use for the preparation of pharmaceutical formulations for the treatment of tumors.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 February 2001 (01.02.2001)

PCT

(10) International Publication Number
WO 01/07418 A2

(51) International Patent Classification[7]: C07D 239/54, 239/56, C07H 15/26, A61K 31/505, A61P 35/00

(21) International Application Number: PCT/EP00/07023

(22) International Filing Date: 21 July 2000 (21.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
FI99A000169 22 July 1999 (22.07.1999) IT

(71) Applicant *(for all designated States except US)*: **BRACCO S.P.A.** [IT/IT]; Via Egidio Folli, 50, I-20134 Milano (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MACCHIA, Bruno** [IT/IT]; Via Lavagna, 15, I-56100 Pisa (IT). **BALSAMO, Aldo** [IT/IT]; Via Consani, 678, I-55100 Lucca (IT). **MACCHIA, Marco** [IT/IT]; Viale Italia, 119, I-57100 Livorno (IT). **DEL TACCA, Mario** [IT/IT]; Lungarno Buozzi, 13, I-56100 Pisa (IT). **DANESI, Romano** [IT/IT]; Viale Ugo Foscolo, 99, I-57100 Livorno (IT).

(74) Agent: **GERVASI, Gemma**; Notarbartolo & Gervasi S.p.A., Corso di Porta Vittoria, 9, I-20122 Milano (IT).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

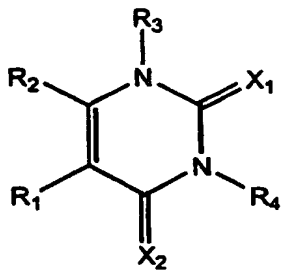
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CERAMIDE ANALOGS, PROCESS FOR THEIR PREPARATION AND THEIR USE AS ANTITUMOR AGENTS

(57) Abstract: The present invention is directed to ceramide analog compounds of general formula (I) the process for their preparation and use for the preparation of pharmaceutical formulations for the treatment of tumors.



(I)

WO 01/07418 A2

CERAMIDE ANALOGS, PROCESS FOR THEIR PREPARATION AND THEIR USE AS ANTITUMOR AGENTS

Field of the invention

The present invention concerns the ceramide analog compounds of the general formula (I) specified below, their corresponding preparation process, and their use in the preparation of pharmaceutical formulations with an antitumor effect.

State of the art

Ceramides are lipids composed of a fatty acid and sphingosine joined together by an amide link; they are generated by sphingomyelin, a sphingolipid occurring in the membranes of eukaryote cells due to the action of the enzyme sphingomyelinase, or they are synthesized by the action of the enzyme ceramide synthetase.

Sphingolipids such as sphingomyelin have always been considered as stable and metabolically inactive structural components of the membranes. It is only in the last decade that it has been demonstrated, instead, that sphingolipids have an active role in the mechanisms regulating cell response to exogenous stimuli, as well as in regulating cell growth, differentiation, transformation and adhesion.

It has also recently been demonstrated that the products of the demolition of sphingolipids, i.e. ceramides and sphingosine, play an important part in regulating the transmission mechanisms of the signals controlled by the membrane

sphingolipids (Teruyuki Sakai et al., *Exp Opin Ther Patents* [1988] 8 [12]: 1673-1682). In particular, the distinctive characteristic of these products seems to be their involvement in the antiproliferative mechanisms of cell regulation, such as cell growth inhibition, the induction of cell differentiation and programmed cell death, or apoptosis.

Apoptosis has recently been the object of numerous studies (e.g. Ross A. Kinloch et al., *TiPS*, Jan 1999 [20]: 35-42), because this phenomenon lends itself to pharmacological "manipulation": in fact, a reduction in the frequency of the onset of cell apoptosis can have severe pathological consequences and facilitate tumor growth, hence the therapeutic potential of all those compounds that are capable of stimulating apoptosis.

From in-depth studies it has emerged that the ceramides in the cell membranes act as intracellular "effectors" of apoptosis, and therefore as potential inhibitors of tumor growth.

In order to boost this capacity of the endogenous ceramides pharmacologically, the ideal strategy seems to be to develop endogenous ceramide analogs that mimic their effects, are stable in relation to metabolization of the sphingosine ceramide and have an inhibitory effect on the ceramidase in order to prevent the generation of sphingosine, which represents a factor that stimulates proliferation, starting from the endogenous sources of ceramides.

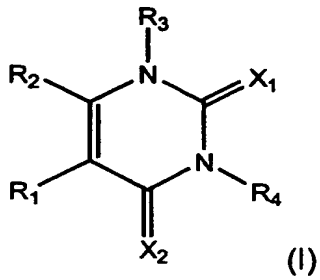
Such ceramide analogs should also have the capacity to penetrate the cell membrane.

There is consequent a need for ceramide analog compounds that are capable of crossing the cell membranes, penetrating inside the cells and mimicking the

various properties of the ceramides, and particularly that of inducing apoptosis in human cancer cells.

Summary of the invention

The Applicant has now surprisingly discovered that the ceramide analog compounds of formula (I):



(I)

wherein:

X_1 and X_2 are selected between O and S;

R_1 and R_2 are selected between $-(CH_2)_{13}CH_3$ and alkyl or alkylene groups with from 2 to 6 carbon atoms, linear or branching, unsubstituted or substituted with one or more substituents selected among aromatic, primary, secondary and tertiary aminic, quaternary ammonium, carboxylic, hydroxylic, polyoxyalkylic and ethereal groups, aminoacids, halogen atoms or saccharidic portions, providing that between R_1 and R_2 only one is always $-(CH_2)_{13}CH_3$,

R_3 and R_4 are selected between H and alkyl or alkylene groups with from 2 to 6 carbon atoms, linear or branching, unsubstituted or substituted with one or more substituents selected among aromatic, primary, secondary and tertiary aminic, quaternary ammonium, carboxylic, hydroxylic, polyoxyalkylic and ethereal groups, aminoacids, halogen atoms or saccharidic portions,

are capable of penetrating inside the biological membranes and effectively inducing apoptosis of the cancer cells.

The compounds of the general formula (I) considered in the present invention have therefore proved suitable for the preparation of pharmaceutical formulations for the treatment of tumors.

The object of the present invention is therefore represented by the compounds of the general formula (I), their corresponding preparation process, and their use in the preparation of pharmaceutical formulations for use in the treatment of tumors.

The characteristics and advantages of the compounds of the general formula (I) according to the present invention will be illustrated in detail in the following description.

DETAILED DESCRIPTION OF THE INVENTION

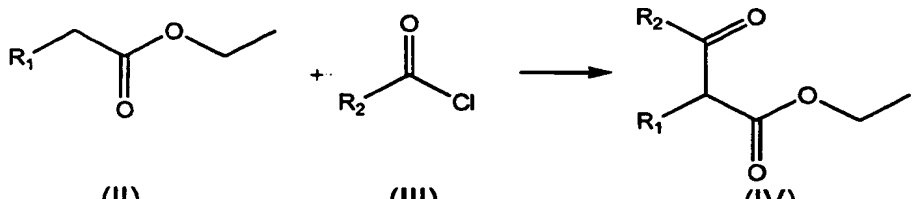
The present invention refers to the compounds of the general formula (I), as defined above. Said compounds (I) have proved capable of penetrating inside the biological membranes and effectively inducing the apoptosis of cancer cells. The following compounds have proved particularly effective and highly cytotoxic:

- compound of formula (I) where X_1= S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = ethyl, and R_3 = R_4 = H [compound **(3)**];
- compound of formula (I) where X_1= X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = ethyl, and R_3 = R_4 = H [compound **(4)**];
- compound of formula (I) where X_1= X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H [compound **(6)**];
- compound of formula (I) where X_1= X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and R_3 = R_4 = H [compound **(10)**];

The present compounds of formula (I) can be conveniently prepared by processes well known in the art. For example, a process for the preparation of the present

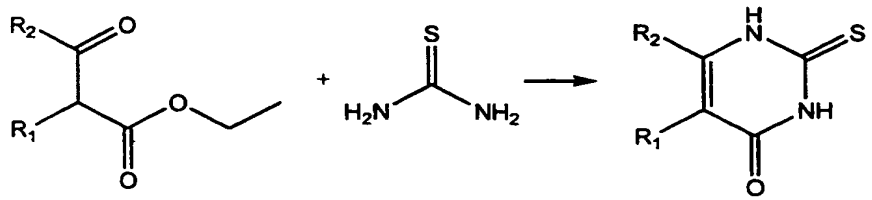
compounds of formula (I) wherein $R_3 = R_4 = H$ includes the following steps:

i) reaction of the ethyl ester (II) with acid chloride (III) to obtain the ß-ketoester of formula (IV):



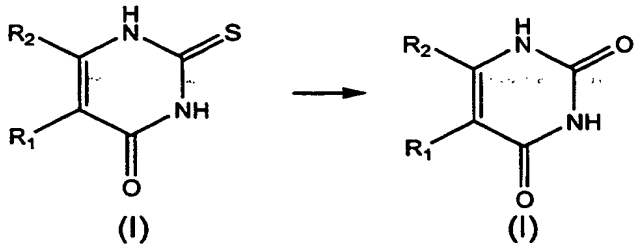
(II) (III) (IV)

ii) reaction of the ß-ketoester of formula (IV) with thiourea (V) to obtain the compound of formula (I) where $X_1 = S$, $X_2 = O$:



(IV) (V) (I)

iii) reaction compound of formula (I) where $X_1 = S$, $X_2 = O$, with refluxed chloroacetic acid to obtain the compound of formula (I) wherein $X_1 = X_2 = O$:



(I) (I)

wherein X_1, X_2, R_1 and R_2 have above-specified meanings.

Step i) of the said process is generally carried out in an organic solvent, such as THF, at a temperature of 0°C. Said reaction is preferably carried out in an inert gas atmosphere.

The reaction product of formula (IV) can be recovered from the reaction mixture by addition of a saturated NH_4Cl solution and subsequent extraction with diethyl ether.

Step ii) of the present process is carried out by means of the addition of thiourea in ethanol and sodium ethoxide on the raw reaction product coming from step i), without the need for any purification. In step ii) temperature is preferably maintained around 90°C. The reaction product is generally recovered from the reaction mixture by acidification at pH 2, e.g. by adding conc. HCl, and filtration of the resulting precipitate, which can be purified, if necessary, by washing with acetone.

The reaction product obtained in step ii) can be further purified by chromatography on silica gel, preferably using a mixture of ethyl acetate and petroleum ether in proportions of 2:1 as an eluant.

Step iii) of the process according to the above procedure is generally carried out by adding chloroacetic acid to the product coming from step ii), e.g. in the form of a 10% aqueous solution, and reflux heated. The crude residue thus obtained can then be purified by washing with absolute ethanol and then with diethyl ether.

The product coming from step iii) can be further purified by chromatography on silica gel, preferably using a mixture of ethyl acetate and hexane in proportions of 1:2 as an eluant.

The present compounds of formula (I) wherein R_3 and/or R_4 are different from H, can be prepared from the ß-ketoester of formula (IV) or from the compounds of formula (I) wherein $R_3 = R_4 = H$, obtained for example as explained above, by means of well-known processes.

Other processes for the preparation of the present formula (I) compounds are disclosed in the examples.

The compounds of formula (I) according to the present invention can be formulated with pharmaceutically acceptable excipients and/or diluents in order to prepare pharmaceutical formulations suitable for the treatment of tumor pathologies.

The following examples are given as a partial illustration of the present invention.

EXAMPLE 1

Preparation of the compound of formula (I) where X_1 = S, X_2 = O, R_2 = $-(CH_2)_{13}CH_3$, R_1 = ethyl, and R_3 = R_4 = H [compound (1)]

A solution prepared by dissolving 0.37 g of ethyl butyrate in 2 ml of anhydrous tetrahydrofuran (THF) is added drop by drop, at a temperature of 0°C and in an argon gas atmosphere, to 1.9 ml of a 2M solution of lithiodiisopropylamine (LDA) in anhydrous THF. After 30 minutes of agitation at 0°C, the reaction mixture is added to a solution obtained by dissolving 1 g of pentadecanol chloride (3.8 mmol) in 5 ml of anhydrous THF, previously cooled to 0°C. The resulting mixture is constantly agitated at room temperature for 12 hours, then added to a saturated solution of NH_4Cl. The organic phase is separated from the aqueous phase, then extracted with diethyl ether. The organic extracts are combined, washed with a saturated aqueous solution of NaCl, dried with anhydrous Na_2SO_4 and then evaporated until dry to provide a crude residue (1.20 g) composed almost exclusively of ß-ketoester (IV) where R_2 = $-(CH_2)_{13}CH_3$ and R_1 = ethyl. [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.83-0.94 (m, 6H), 1.07 (t, 3H, *J* = 7.4 Hz), 1.15-1.36 (m, 24H), 1.81-2.02 (m, 2H), 2.11-2.57 (m, 2H), 3.34 (t, 1H, *J* = 7.3 Hz), 4.15 (q, 2H, *J* = 7.3 Hz). MS m/e 340 M^+].

The resulting crude residue (1.20 g) containing the ß-ketoester (IV) where R_2 = –

$(CH_2)_{13}CH_3$ and R_1 = ethyl, is dissolved in 20 ml of absolute ethanol and then added to 3.61 g of thiourea (47.5 mmol) and 6.47 g of sodium ethoxide (95.1 mmol). The mixture is agitated for 60 minutes at 90°C. After cooling to room temperature, the reaction mixture is filtered and the filtrate is evaporated until dry; the residue thus obtained is then restored with a mixture of water and THF in proportions of 10:1 until it has become completely soluble. The solution, cooled to 0°C, is acidified to pH 2 with conc. HCl; the precipitate that develops is filtered and washed with small quantities of acetone and provides a crude residue that is purified by chromatography on silica gel using ethyl acetate and petroleum ether in proportions of 2:1 as an eluant, finally obtaining 290 mg (0.82 mmol; yield = 26%) of the required compound of formula (I) (m.p. = 167-169°C; [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.89 (t, 3H, *J* = 6.2 Hz), 1.09 (t, 3H, *J* = 7.4 Hz), 1.17-1.36 (m, 24H), 2.34-2.49 (m, 4H), 8.88 (br, 1H, D_2O exchangeable), 9.81 (br, 1H, D_2O exchangeable); MS m/e 352 M^+).

EXAMPLE 2

Preparation of the compound of formula (I) where X_1 = X_2 = O, R_2 = $-(CH_2)_{13}CH_3$, R_1 = ethyl, and R_3 = R_4 = H [compound (2)]

160 mg (0.45 mmol) of the product (1) obtained as described in Example 1 are added to 11.4 ml of a 10% aqueous solution of chloroacetic acid and the mixture thus obtained is reflux heated for 12 hours. The resulting precipitate is then filtered, washed first with absolute ethanol, then with diethyl ether, to obtain a crude residue that, after purification by chromatography on silica gel using a mixture of ethyl acetate and hexane in proportions of 1:2 as an eluant, gave rise to 48 mg (0.14 mmol, yield = 32%) of the required pure compound (m.p. = 132-

134°C; [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.2 Hz), 1.06 (t, 3H, *J* = 7.4 Hz), 1.15-1.36 (m, 24H), 2.31-2.49 (m, 4H), 9.06 (br, 1H, D_2O exchangeable), 9.89 (br, 1H, D_2O exchangeable); MS m/e 336 M^+).

EXAMPLE 3

Preparation of the compound of formula (I) wherein X_1= S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = ethyl, and R_3 = R_4 = H [compound (3)]

A solution obtained by dissolving 1 g of ethyl palmitate (3.52 mmol) in 3 ml of anhydrous THF is added drop by drop, at a temperature of 0°C in an argon gas atmosphere, to 2.1 ml of a 2M solution of lithiodiisopropylamine (LDA) in anhydrous THF. After 30 minutes of agitation at 0°C, the reaction mixture is added to a solution obtained by dissolving 2.39 g (4.23 mmol) of propionyl chloride in 5 ml of anhydrous THF. The resulting mixture is constantly agitated at room temperature for 12 hours, then added to a saturated solution of NH_4Cl. The organic phase is separated from the aqueous phase, then extracted with diethyl ether. The organic extracts are combined, washed with a saturated aqueous solution of NaCl, dried with anhydrous Na_2SO_4 and then evaporated until dry to provide a crude residue (1.31 g) composed almost exclusively of ß-ketoester (IV) where R_1 = –$(CH_2)_{13}CH_3$ and R_2 = ethyl. [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.79-0.92 (m, 6H), 1.11 (t, 3H, *J* = 7.6 Hz), 1.17-1.39 (m, 24H), 1.48-1.62 (m, 2H), 2.26 (q, 2H, *J* = 7.6 Hz), 3.36 (t, 1H, *J* = 7.3 Hz), 4.15 (q, 2H, *J* = 7.2 Hz); MS m/e 340 M^+].

1.31 g of the resulting crude residue containing the ß-ketoester (IV) where R_1 = –$(CH_2)_{13}CH_3$ and R_2 = ethyl, is dissolved in 20 ml of absolute ethanol and then added to 4.01 g of thiourea (52.8 mmol) and 7.18 g of sodium ethoxide (105.6 mmol). The mixture is agitated for 60 minutes at 90°C. After cooling to room

temperature, the reaction mixture is filtered and the filtrate is evaporated until dry; the residue thus obtained is then treated with a mixture of water and THF in proportions of 10:1 until it has become completely soluble. The solution is cooled to 0°C and acidified to pH 2 with conc. HCl; the precipitate that develops due to acidification is filtered and washed with small quantities of acetone and provides a crude residue that is purified by chromatography on silica gel using ethyl acetate and petroleum ether in proportions of 2:1 as an eluant, finally obtaining 310 mg (0.88 mmol; yield = 25%) of a product that coincides with the required pure compound **3** (m.p. = 100-102°C; [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.01 (t, 3H, *J* = 7.4 Hz), 1.18-1.38 (m, 24H), 2.35 (t, 2H, *J* = 7.4 Hz), 2.48 (q, 2H, *J* = 7.6 Hz), 9.08 (br, 1H, D_2O exchangeable), 9.73 (br, 1H, D_2O exchangeable); MS m/e 352 M^+).

EXAMPLE 4

Preparation of the compound of formula (I) wherein $X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ [compound (4)]

160 mg (0.45 mmol) of the compound (**3**) obtained as described in Example 3 are added to 11.4 ml of a 10% aqueous solution of chloroacetic acid and the mixture thus obtained is reflux heated for 12 hours. The resulting precipitate is then filtered, washed first with absolute ethanol, then with diethyl ether, to obtain a crude residue that, after purification by chromatography on silica gel using a mixture of ethyl acetate and hexane in proportions of 1:2 as an eluant, gave rise to 57 mg (0.17 mmol, yield = 38%) of the compound (**4**) (m.p. = 110-112°C; [^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.89 (t, 3H, *J* = 6.4 Hz), 1.02 (t, 3H, *J* = 7.4 Hz), 1.12-1.42 (m, 24H), 2.34 (t, 2H, *J* = 7.2 Hz), 2.49 (q, 2H, *J* = 7.6 Hz), 9.15 (br, 1H, D_2O

exchangeable), 9.53 (br, 1H, D_2O exchangeable); MS m/e 336 M^+).

EXAMPLE 5

Preparation of the compound of formula (I) wherein X_1 = S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H [compound (5)]

Compound (**5**) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 366 M^+.

EXAMPLE 6

Preparation of the compound of formula (I) wherein X_1 = X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H [compound (6)]

Compound (**6**) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 350 M^+.

EXAMPLE 7

Preparation of the compound of formula (I) wherein X_1 = S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = *n*-butyl, and R_3 = R_4 = H [compound (7)]

Compound (**7**) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 380 M^+.

EXAMPLE 8

Preparation of the compound of formula (I) wherein X_1 = X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = *n*-butyl, and R_3 = R_4 = H [compound (8)]

Compound (**8**) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 364 M^+.

EXAMPLE 9

Preparation of the compound of formula (I) wherein X_1 = S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and R_3 = R_4 = H [compound (9)]

Compound (**9**) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 380 M^+.

EXAMPLE 10

Preparation of the compound of formula (I) wherein X_1 = X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 =i-butyl, and R_3 = R_4 = H [compound (10)]

Compound (**10**) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 364 M^+.

EXAMPLE 11

Preparation of the compound of formula (I) wherein X_1 = S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = neopentyl, and R_3 = R_4 = H [compound (11)]

Compound (**11**) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 394 M^+.

EXAMPLE 12

Preparation of the compound of formula (I) wherein X_1 = X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = neopentyl, and R_3 = R_4 = H [compound (12)]

Compound (**12**) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 378 M^+.

EXAMPLE 13

Preparation of the compound of formula (I) wherein X_1 = S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$, R_2 = 2-phenyl-ethyl, and R_3 = R_4 = H [compound (13)]

Compound (**13**) was prepared following a procedure similar to the one described in Example 3, obtaining a product which resulted in: MS m/e 428 M^+.

EXAMPLE 14

Preparation of the compound of formula (I) wherein X_1 = X_2 = O, R_1 = –

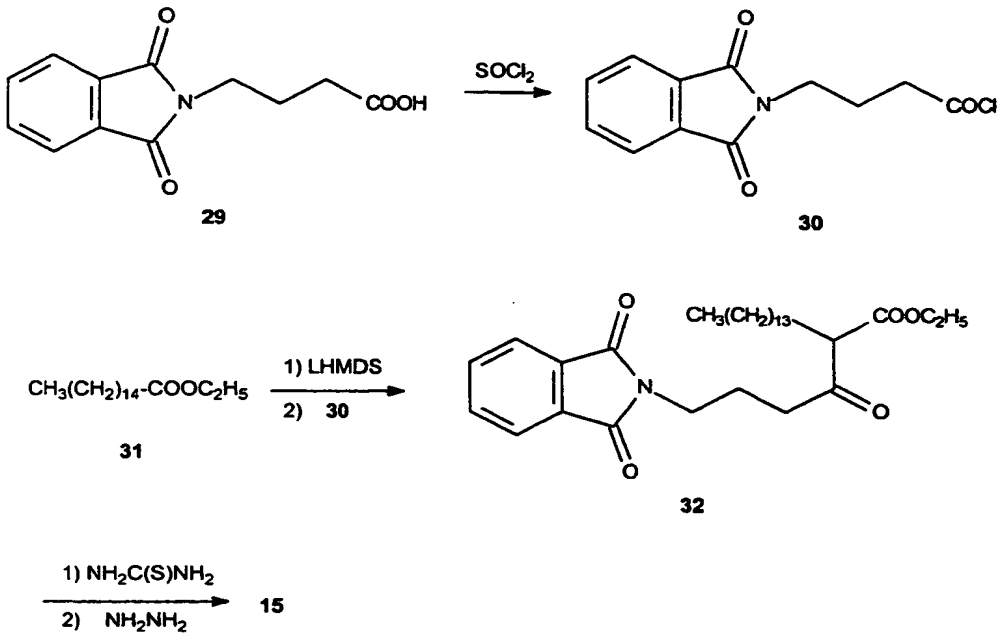
$(CH_2)_{13}CH_3$, R_2 = 2-phenyl-ethyl, and $R_3 = R_4$ = H [compound (14)]

Compound (**14**) was prepared following a procedure similar to the one described in Example 4, obtaining a product which resulted in: MS m/e 412 M^+.

EXAMPLE 15

Preparation of the compound of formula (I) wherein X_1 =S, X_2 = O, R_1 = – $(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3NH_2$ and $R_3 = R_4$ = H [compound (15)]

Scheme 1



Compound (**15**) was prepared following the procedure described in the above Scheme 1.

Synthesis of β-ketoester (**32**). 2.4 g (10 mmol) of 4-phthalimidoburyric acid (**29**) (prepared as described in G. Talbot, R. Gaudry, L. Berlinguet *Can. J. Chem.* **1958**, *36*, 593-596) was dissolved in 7.5 ml of $SOCl_2$ and the mixture was refluxed under nitrogen for 3 hours. Excess of $SOCl_2$ was then removed under a nitrogen flow and the resulting acid chloride (**30**) was used in the next step without further

purification. Separately, a solution of ethyl palmitate (**31**) (1.47 g, 5.16 mmol) in anhydrous THF (6.5 ml) was slowly added to a 1.0 M solution of lithium bis(trimethylsilyl)amide (LHMDS) in THF (6.2 ml, 6.2 mmol) cooled at -20 °C and the resulting mixture was stirred for additional 20 minutes. Acid chloride (**30**) previously prepared as described above, was dissolved in anhydrous THF (10 ml), cooled at -20 °C, and added via cannula to the solution containing (**31**) and LHMDS at the same temperature. The mixture was stirred at -20 °C for 30 minutes and then at room temperature for 2 hours. The reaction was quenched with a saturated aqueous solution of ammonium chloride and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-ethyl acetate (8:2) as the eluant, to obtain 0.95 g (1.9 mmol, 37% yield) of pure β-ketoester (**32**) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.4 Hz), 1.23 (t, 3H, *J* = 7.3 Hz), 1.24 (bs, 24H), 2.05 (quintet, 2H, *J* = 7.2 Hz), 2.24-2.34 (m, 4H), 2.51 (t, 2H, *J* = 7.6 Hz), 3.78 (t, 1H, *J* = 6.9 Hz), 4.12 (q, 2H, *J* = 7.1 Hz), 7.69-7.73 (m, 2H), 7.82-7.87 (m, 2H); MS (FAB$^+$) m/z 500 $(M+H)^+$.

Synthesis of thiouracil (**15**): β-Ketoester (**32**) (0.12 g, 0.24 mmol) was dissolved in 2 ml of absolute ethanol. Thiourea (0.024 g, 0.33 mmol) and potassium *t*-butoxyde (0.028 g, 0.25 mmol) were added and the resulting mixture was refluxed for 5 hours. The mixture was then cooled to room temperature and the solvent was removed under vacuum. The residue was treated with 20 ml of water and neutralized with an aqueous solution of acetic acid 0.5 N. The product was extracted with ethyl acetate and the organic layer was washed with brine, dried

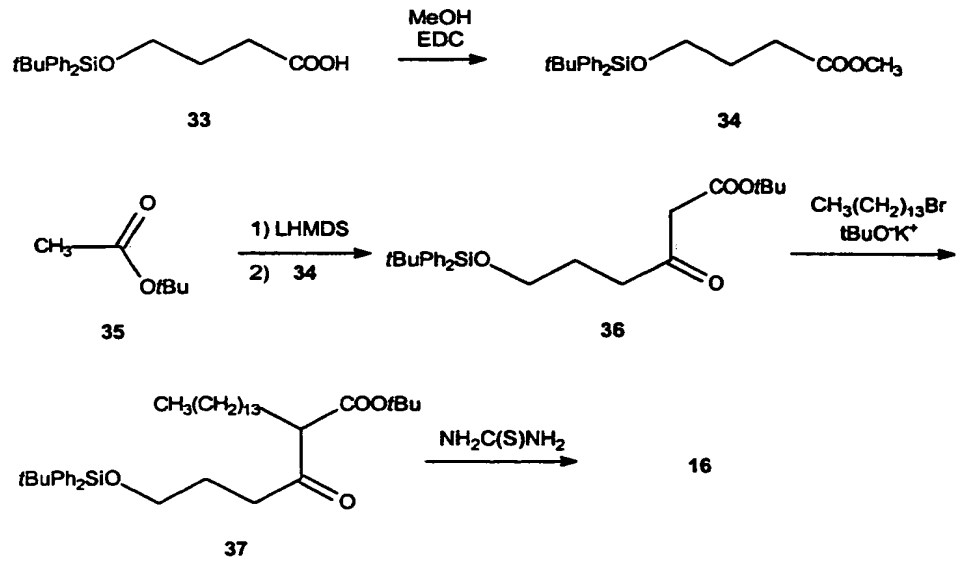
over anhydrous sodium sulfate and concentrated under vacuum. The crude residue was then redissolved in 3 ml of ethanol, treated with 0.06 ml of hydrazine monohydrate (1.3 mmol), and the mixture was refluxed overnight. The resulting suspension was cooled to room temperature. The white solid was collected by filtration, washed with small portions of ethyl acetate, and dried under vacuum, to give 51 mg (0.13 mmol, 54% yield) of product (**15**): m.p. 123-125 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, 3H, *J* = 6.4 Hz), 1.26 (bs, 24H), 1.77 (m, 2H), 2.29-2.45 (m, 6H), 8.87 (bs, 1H), 9.19 (bs, 1H); MS (FAB$^+$) m/z 381 $(M+H)^+$.

EXAMPLE 16

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OSiPh_2t$-Bu and R_3 = R_4 = H [compound (16)]

Compound (**16**) was prepared following the procedure described in the following

Scheme 2



Scheme 2.

Synthesis of methyl ester (**34**). A solution of acid (**33**) (1.15 g, 3.36 mmol) (prepared as in: A.G.M. Barrett, J.A. Flygare *J. Org. Chem.* **1991**, *56*, 638-642) in methanol (25 ml) was treated with 1.62 g (8.44 mmol) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC). The resulting solution was stirred under nitrogen at room temperature for 3.5 hours. The solvent is then removed under vacuum and the residue was diluted with chloroform (100 ml) and water (50 ml). The organic layer was washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-ethyl acetate (9:1) as the eluant, to obtain 0.59 g (1.6 mmol, 49% yield) of pure ester (**34**) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.05 (s, 9H), 1.88 (tt, 2H, *J* = 7.7, 5.9 Hz), 2.47 (t, 2H, *J* = 7.5 Hz), 3.66 (s, 3H), 3.68 (t, 2H, *J* = 6.0 Hz), 7.37-7.42 (m, 6H), 7.63-7.68 (m, 4H).

Synthesis of β-ketoester (**36**). A solution of *t*-butyl acetate (**35**) (4.24 g, 36.5 mmol) in anhydrous THF (40 ml) previously cooled at -78 °C was added drop by drop via cannula under argon to a 1M solution of LHMDS in THF (51.5 ml, 51.5 mmol). To the resulting solution, previously stirred at the same temperature for 30 minutes, was added drop by drop via cannula another solution of methyl ester (**34**) (4.07 g, 11.4 mmol) in anhydrous THF (20 ml) at -78 °C. The reaction mixture was stirred under argon for 20 minutes at the same temperature, and then 3 more hours at room temperature. The reaction was quenched with 400 ml of saturated aqueous solution of ammonium chloride and extracted with diethyl ether (2 x 300 ml). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-diethyl ether (8:2) as the eluant, to obtain 2.8 g (6.4 mmol, 56%

yield) of pure β-ketoester (**36**) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.04 (s, 9H), 1.46 (s, 9H), 1.84 (quintet, 2H, *J* = 6.7 Hz), 2.66 (t, 2H, *J* = 7.3 Hz), 3.34 (s, 2H), 3.67 (t, 2H, *J* = 6.0 Hz), 7.37-7.43 (m, 6H), 7.62-7.67 (m, 4H); MS (FAB^+) m/z 441 $(M+H)^+$, 385 $(M+H\text{-isobutene})^+$.

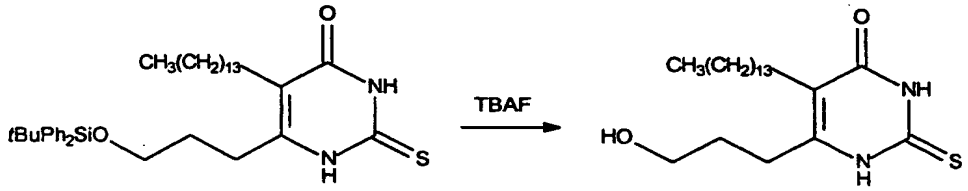
Synthesis of alkylated β-ketoester (**37**). A solution of β-ketoester (**36**) (2.79 g, 6.34 mmol) in anhydrous 1,2-dimethoxyethane (DME) (17 ml) was added to a solution of potassium *tert*-butoxide (0.85 g, 6.97 mmol) in anhydrous DME (7 ml). The resulting solution was stirred at room temperature for 20 minutes, after which time 1.7 ml (1.6 g, 5.7 mmol) of 1-bromotetradecane were added. The reaction mixture was stirred at 80 °C for 2 hours. The reaction was quenched with 150 ml of a saturated aqueous solution of ammonium chloride and extracted with diethylether (2 x 300 ml). The organic layer was washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-diethyl ether (9:1) as the eluant, to obtain 1.16 g (1.82 mmol, 32% yield) of pure mono-alkylated product (**37**) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, 3H, *J* = 6.2 Hz), 1.04 (s, 9H), 1.25 (bs, 24H), 1.43 (s, 9H), 1.76-1.89 (m, 4H), 2.64 (td, 2H, *J* = 7.3, 4.4 Hz), 3.13 (t, 1H, *J* = 7.3 Hz), 3.66 (t, 2H, *J* = 6.0 Hz), 7.34-7.43 (m, 6H), 7.62-7.67 (m, 4H); MS (FAB^+) m/z 581 $(M+H\text{-isobutene})^+$, 563 $(M\text{-}t\text{BuO})^+$.

Synthesis of thiouracil (**16**). A solution containing alkylated β-ketoester (**37**) (1.16 g, 1.82 mmol) in absolute ethanol (24 ml) in a screw-cap sealed vial was treated first with 0.19 g (2.6 mmol) of thiourea and then with 0.25 g (2.0 mmol) of potassium *tert*-butoxide. The resulting solution was stirred at 100 °C for 6 hours. The solvent was then removed under vacuum. The residue was diluted with water

and neutralized to pH = 6-7 with 0.5 N acetic acid. The product was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-diethyl ether (8:2) as the eluant, to obtain 0.52 g (0.84 mmol, 46% yield) of pure thiouracil product (**16**) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.2 Hz), 1.10 (s, 9H), 1.24 (bs, 24 H), 1.74 (quintet, 2H, *J* = 6.8 Hz), 2.34 (t, 2H, *J* = 7.4 Hz), 2.60 (t, 2H, *J* = 7.5 Hz), 3.74 (t, 2H, *J* = 5.8 Hz), 7.40-7.46 (m, 6H), 7.66-7.70 (m, 4H), 9.29 (bs, 1H), 9.55 (bs, 1H); MS (FAB$^+$) m/z 547 (M+H-NHC(S)NH)$^+$.

EXAMPLE 17

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OH$ and R_3 = R_4 = H [compound (17)]



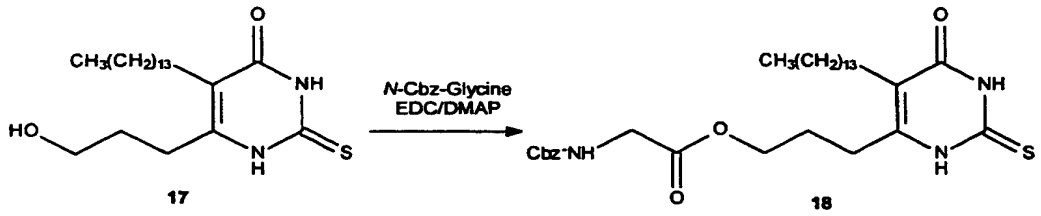
Scheme 3

According to the above Scheme 3 the silyl ether **16** (0.16 g, 0.26 mmol) was treated with 0.8 ml of a 1M solution of tetrabutylammonium fluoride (TBAF) in THF (0.8 mmol) under argon at room temperature for 2 hours. The solvent was then removed under vacuum and the residue was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude residue was purified by silica gel column chromatography using hexane-ethyl acetate (1:1) as the eluant, to obtain 0.079 g (0.21 mmol, 81% yield) of pure deprotected alcohol (**17**) as a white solid: m.p. 128-

130 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (t, 3H, *J* = 6.7 Hz), 1.25 (bs, 24H), 1.91 (quintet, 2H, *J* = 6.1 Hz), 2.37 (pseudo t, 2H, *J* = 7.4 Hz), 2.67 (pseudo t, 2H, *J* = 6.3 Hz), 3.84 (t, 2H, *J* = 5.6 Hz), 9.16 (bs, 1H), 10.52 (bs, 1H); MS (EI, 70 eV) m/z 382 $(M)^+$, 365 $(M\text{-}OH)^+$, 323 $(M\text{-}NHC{=}S)^+$.

EXAMPLE 18

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OC(O)CH_2NH$-Cbz and R_3 = R_4 = H [compound (18)]



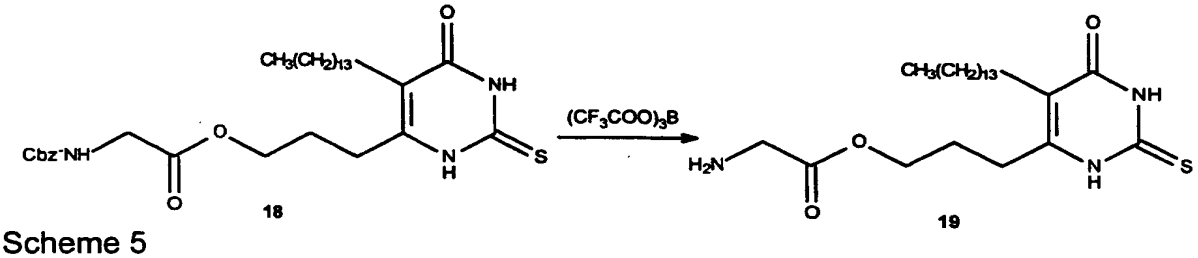
Scheme 4

According to scheme 4 a solution of the alcohol (**17**) (0.038 g, 0.099 mmol) in anhydrous THF (2.5 ml) was sequentially treated with 0.031 g (0.15 mmol) of *N*-carbobenzyloxyglycine (*N*-Cbz-Gly), 0.034 g (0.18 mmol) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), and 0.0012 g (0.0096 mmol) of 4-(dimethylamino)pyridine (DMAP). The mixture was stirred at room temperature for 5 hours under argon. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography using hexane-ethyl acetate (1:1) as the eluant, to obtain 0.052 g (0.091 mmol, 92% yield) of product (**18**) as a thick syrup: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, J = 6.6 Hz), 1.25 (bs, 24H), 1.91 (m, 2H), 2.31 (t, 2H, J = 7.7 Hz), 2.47 (t, 2H, J = 7.7 Hz), 4.07 (d, 2H, J = 5.9 Hz), 4.27 (t, 2H, J = 5.2 Hz), 5.24 (s, 2H), 5.52 (t, 1H, J = 5.8 Hz), 7.31-7.38 (m, 5H), 10.09 (bs, 1H), 10.85 (bs 1H); MS (FAB^+) m/z 574

$(M+H)^+$, 532 $(M-C(S)+H)^+$.

EXAMPLE 19

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OC(O)CH_2NH_2$ and R_3 = R_4 = H [compound (19)]

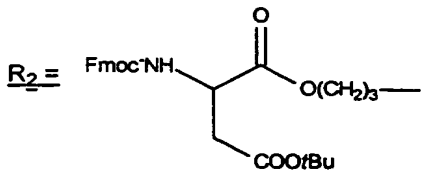


Scheme 5

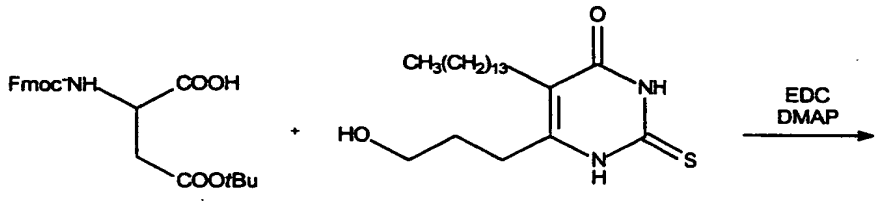
According to the above scheme 5 a solution of Cbz-protected compound (**18**) (0.032 g, 0.055 mmol) in trifluoroacetic acid (1 ml) was treated with 0.22 mmol of freshly prepared boron tris(trifluoroacetate) (prepared as reported in: J. Pless, W. Bauer *Angew. Chem. Int. Ed.* **1973**, *12*, 147-148) at 0 °C under argon. The mixture was stirred for 1 hour at the same temperature and overnight at room temperature. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography using a mixture dichloromethane : acetone 7 : 3 as the eluant, to obtain 0.020 g (0.045 mmol, 82% yield) of product (**19**) as a thick syrup: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.4 Hz), 1.25 (bs, 24H), 1.72 (m, 2H), 2.32 (m, 2H), 2.63 (m, 2H), 3.61 (t, 2H, *J* = 7.0 Hz), 4.30 (t, 2H, *J* = 6.6 Hz); MS (FAB^+) m/z 365 $(M-NHC(S)NH)^+$.

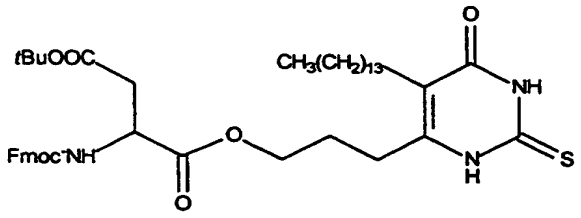
EXAMPLE 20

Preparation of the compound of formula (I) where X_1=S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$,



and R_3 = R_4 = H [compound (20)]





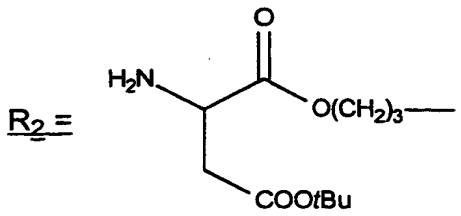
Scheme 6

According to the above scheme 6 a solution of alcohol (**17**) (0.120 g, 0.314 mmol) in anhydrous THF (10 ml) was treated sequentially with *N*-(9-Fluorenylmethoxycarbonyl)-L-aspartic acid *tert*-butyl ester (**38**) (0.194 g, 0.471 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) (0.108 g, 0.562 mmol) and 4-(dimethylamino)pyridine (DMAP) (0.0077 g, 0.063 mmol). The mixture was stirred under argon at room temperature for 5 hours. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography (hexane - ethyl acetate 1:1) to afford 0.24 g (0.31 mmol, 98 % yield) of product (**20**) as a syrup: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.3 Hz), 1.25 (bs, 24H), 1.46 (s, 9H), 1.93 (m, 2H), 2.30 (m, 2H), 2.49 (m, 2H),

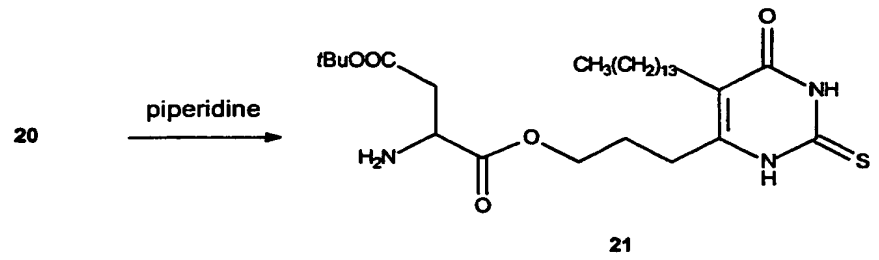
2.80 (dd, 1H, *J* = 16.6, 4.8 Hz), 2.93 (dd, 1H, *J* = 16.6, 5.0 Hz), 4.24-4.33 (m, 2H), 4.48-4.54 (m, 2H), 4.67-4.72 (m, 1H), 5.97 (d, 1H), 7.29-7.43 (m, 5H), 7.62 (d, 2H, *J* = 7.2 Hz), 7.76 (d, 2H, *J* = 7.2 Hz), 9.59 (bs, 1H), 10.58 (bs, 1H).

EXAMPLE 21

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$,



and R_3 = R_4 = H [compound (21)]

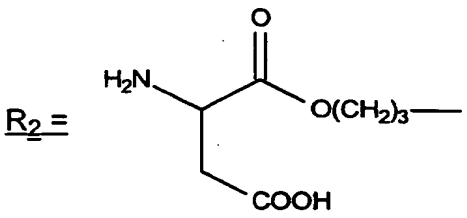


Scheme 7

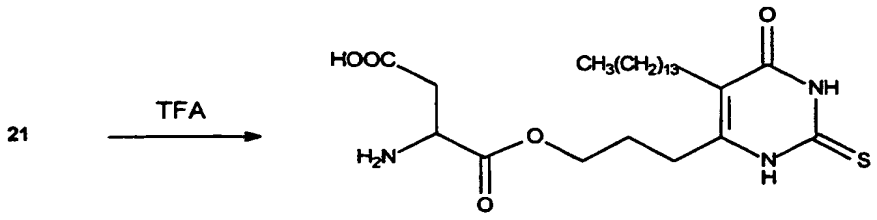
According to the above scheme 7 a solution of Fmoc-protected product (**20**) (0.120 g, 0.155 mmol) in anhydrous dichloromethane (5 ml) was treated with 0.020 g of piperidine (0.23 mmol). The mixture was stirred at room temperature for 30 minutes. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography (hexane - ethyl acetate 3:7) to afford 0.040 g (0.072 mmol, 47 % yield) of product (**20**) as a syrup: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.6 Hz), 1.25 (bs, 24H), 1.46 (s, 9H), 1.94 (m, 2H), 2.33 (t, 2H, *J* = 7.2 Hz), 2.56 (t, 2H, *J* = 7.7 Hz), 2.76 (d, 2H, *J* = 5.9 Hz), 3.94 (t, 1H, *J* = 5.8 Hz), 4.21-4.30 (m, 2H).

EXAMPLE 22

Preparation of the compound of formula (I) where X_1=S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$,



and R_3 = R_4 = H [compound (22)]



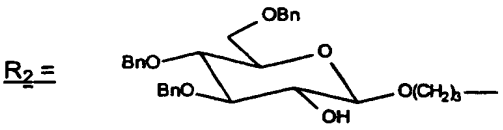
22

Scheme 8

According to the above scheme 8 the *tert*-Butyl ester (**21**) (0.020 g, 0.040 mmol) was treated with 0.2 ml of a 1:1 mixture of trifluoroacetic acid and dichloromethane. The mixture was stirred at room temperature for 1 hour. The solvent was removed under vacuum and the residue was purified by silica gel column chromatography (acetone - methanol, variable ratios from 100:0 to 50:50) to afford 0.012 g (0.021 mmol, 54 % yield) of product (**20**) as a syrup: ^{1}H NMR (CD_3OD, 200 MHz) δ 0.89 (t, 3H, *J* = 6.8 Hz), 1.29 (bs, 24H), 1.97 (m, 2H), 2.35 (m, 2H), 2.57 (m, 2H), 2.82 (m, 2H), 4.16-4.44 (m, 3H).

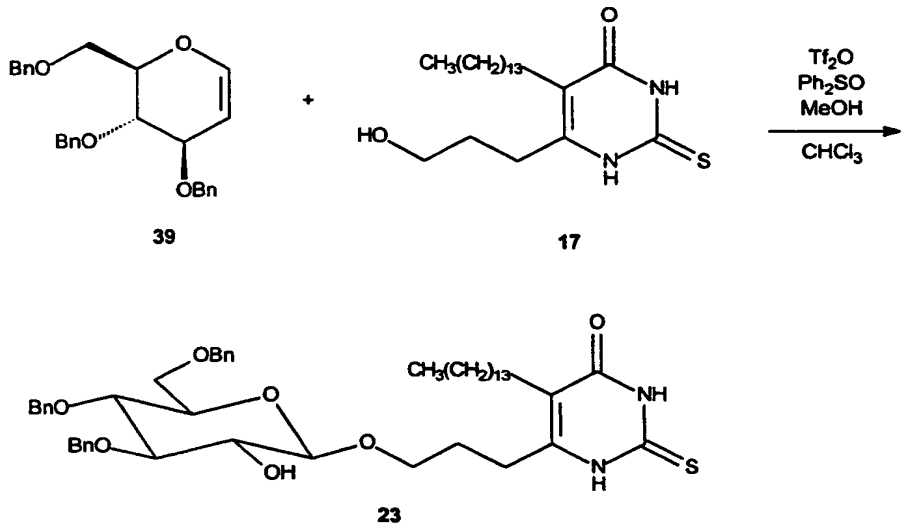
EXAMPLE 23

Preparation of the compound of formula (I) where X_1 =S, X_2 = O, R_1 = –$(CH_2)_{13}CH_3$,



and R_3 = R_4 = H [compound (23)]

Scheme 9



wherein Bn is benzyl.

Glucose derivative (**23**) was prepared following a general procedure for direct glycosilation of alcohols with glucal donor (**39**) (as reported in: V. Di Bussolo, Y.-J. Kim, D.Y. Gin *J. Am. Chem. Soc.* **1998**, *120*, 13515-13516), as reported above in scheme 9.

Trifluoromethanesulfonic anhydride (Tf_2O) (0.030 ml, 0.18 mmol) was added to a solution of tri-O-benzil-D-glucal (**39**) (0.050 g, 0.12 mmol), diphenylsulfoxide (0.073 g, 0.36 mmol) and 2,4,6-tri-*t*-butylpyridine (0.104 g, 0.42 mmol) in dry chloroform (5 ml) (distilled over P_2O_5) at - 40 °C. The reaction mixture was stirred at this temperature for 1 hour. Methanol (0.005 ml, 0.12 mmol) and triethylamine

(0.050 ml, 0.36 mmol) were added sequentially at -40 °C and the reaction mixture was stirred at this temperature for 30 minutes, then at 0 °C for 1 hour and at room temperature for 1 hour. A solution of alcohol derivative (**17**) (0.065 g, 0.17 mmol) in dry chloroform (4 ml) was added at 0 °C, via cannula. Zinc chloride (0.24 ml, 1.0 M in diethyl ether, 0.24 mmol) was added at the same temperature, then the temperature was slowly warmed to room temperature and the reaction mixture stirred at this temperature for 12 hours. The reaction was diluted with chloroform (15 ml) and washed sequentially with saturated aqueous sodium bicarbonate solution (2 x 15 ml) and a saturated aqueous solution of sodium chloride (15 ml). The organic layer was dried (Na_2SO_4) and concentrated, the residue was purified by silica gel column chromatography (hexane-ethyl acetate 6:4) to afford product (**23**) (0.055 g, 0.067 mmol, 56% yield) as a colourless oil: ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, *J* = 6.3 Hz), 1.25 (bs, 24 H), 1.88 (quintet, 2H, *J* = 6.4 Hz), 2.44 (pseudo *t*, 2H, *J* = 7.5 Hz), 2.65 (t, 2H, *J* = 6.6 Hz), 3.70-3.66 (m, 8H), 4.47 (d, 1H, *J* = 10.6 Hz), 4.52 (d, 1H, *J* = 12.1 Hz), 4.65 (d, 1H, *J* = 12.1 Hz), 4.80 (d, 1H, *J* = 10.8 Hz), 4.86 (d, 1H, *J* = 11.4 Hz), 4.92 (d, 1H, *J* = 11.2 Hz), 5.12 (d, 1H, *J* = 9.2 Hz), 7.09-7.35 (m, 15H), 9.61 (bs, 1H), 11.29 (bs, 1H); MS (FAB$^+$) m/z 815 $(M+H)^+$.

EXAMPLE 24

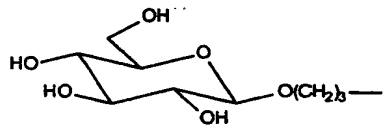
Preparation of the compound of formula (I) where X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = ethyl, R_3 = $-CH_2COOC_2H_5$, and R_4 = H [compound (24)]

Anhydrous $(NH_4)_2SO_4$ (0.0013 g, 0.011 mmol) and 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (0.75 ml, 3.41 mmol) were added, under argon atmosphere, to compound (**3**) (0.05 g, 0.14 mmol). The resulting suspension was heated at 130 °C and stirred at this temperature for 6 hours. The mixture was then

concentrated at room temperature under a flux of argon. Anhydrous THF (3 ml) was added, and the resulting solution was stirred at - 45°C. Trimethylsilyl triflate (TMS triflate) (0.03 ml, 0.145 mmol) and ethyl bromoacetate (0.046 g, 0.027 mmol) were sequentially added and the mixture was stirred at - 45 °C for 3 hours, then at room temperature for 1 hour. Saturated aqueous $NaHCO_3$ (3 ml) was added and THF was removed under vacuum. The residue was diluted with H_2O (20 ml) and extracted with ethyl acetate (3 x 10 ml). The organic layer was dried with Na_2SO_4 anhydrous, and concentrated to dryness. The residue was purified by semi-preparative thin-layer column chromatography (hexane/ethyl acetate 7:3) to afford product (**24**) (0.010 g, 0.023 mmol, 16% yield) as a colourless oil: ^1H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, *J* = 6.6 Hz), 1.17 (t, 3H, *J* = 7.2 Hz), 1.25-1.43 (m, 27H), 2.44 (pseudo t, 2H, *J* = 7.2 Hz), 2.54 (t, 2H, *J* = 7.5 Hz), 3.91 (s, 2H); 4.21 (q, 2H, *J* = 7.3 Hz), 10.88 (bs, 1H);); MS (FAB^+) m/z 439 $(M+H)^+$.

According to procedures analogues to those above reported, the following compounds of formula (I) were prepared:

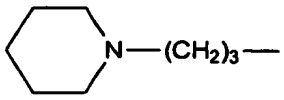
- compound (I) wherein X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$,

R_2 = 

and R_3 = R_4 = H [compound (**25**)];

- compound (I) wherein X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3Br$ and R_3 = R_4 = H [compound (**26**)];

- compound (I) wherein X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$,

$R_2 =$ 

and $R_3 = R_4 = H$ [compound (**27**)];

- compound (I) wherein $X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, $R_2 = -(CH_2)_3N(C_2H_5)_3^+Br^-$ and $R_3 = R_4 = H$ [compound (**28**)].

CYTOTOXICITY TEST

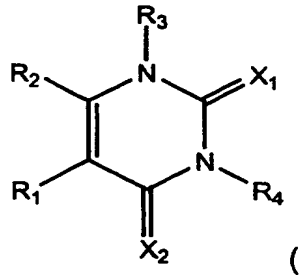
The cytotoxicity of the compounds synthesized 1 - 28 was assessed using a human leukemia cell line called CCRF/CEM. The CCRF/CEM cells were cultured in a culture medium containing RPMI 1640 (90%), bovine fetal sera (10%) and interleukin-2 (100 U/ml). The cytotoxicity assay was performed on 104 CCRF/CEM cells seeded in 35 mm wells in 2 ml of culture medium. The cells were treated with the compounds under consideration for 72 hours and at the end of the period of exposure their number was counted and compared with that of control cells treated with C_2-ceramide in order to establish the percentage of growth inhibition. The concentration capable of inhibiting 50% of cell growth was calculated by non-linear regression of the experimental data as described in M. Macchia, N. Jannitti, G.B. Gervasi, R. Danesi, *J Med Chem*, (1996) 39 (7): 1352-1356.

The resulting values of IC_{50} expressed in μM are given in the following table:

Compound	IC_{50} (μM)
controls	31.6
(3)	1.7
(4)	6.3
(6)	0.97
(9)	13.2
(10)	8.7
(11)	20
(12)	29.1
(13)	20.7
(14)	15.6

Claims

1. Compounds of general formula (I)



(I)

where

X_1 and X_2 are selected between O and S;

R_1 and R_2 are selected between $-(CH_2)_{13}CH_3$ and alkyl or alkylene groups with from 2 to 6 carbon atoms, linear or branching, unsubstituted or substituted with one or more substituents selected among aromatic, primary, secondary and tertiary aminic, quaternary ammonium, carboxylic, hydroxylic, polyoxyalkyl and ethereal groups, aminoacids, halogen atoms or saccharidic portions, providing that between R_1 and R_2 only one is always $-(CH_2)_{13}CH_3$,

R_3 and R_4 are selected between H and alkyl or alkylene groups with from 2 to 6 carbon atoms, linear or branching, unsubstituted or substituted with one or more substituents selected among aromatic, primary, secondary and tertiary aminic, quaternary ammonium, carboxylic, hydroxylic, polyoxyalkyl and ethereal groups, aminoacids, halogen atoms or saccharidic portions.

2. The compounds of general formula (I) according to claim 1, where:

$X_1 = S$, $X_2 = O$, R_1 = ethyl, $R_2 = -(CH_2)_{13}CH_3$, and $R_3 = R_4 = H$ (compound **1**);

$X_1 = X_2 = O$, R_1 = ethyl, $R_2 = -(CH_2)_{13}CH_3$, and $R_3 = R_4 = H$ (compound **2**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ (compound **3**);

$X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ (compound **4**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H (compound **5**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and R_3 = R_4 = H (compound **6**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-butyl, and R_3 = R_4 = H (compound **7**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *n*-butyl, and R_3 = R_4 = H (compound **8**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and R_3 = R_4 = H (compound **9**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and R_3 = R_4 = H (compound **10**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = neopentyl, and R_3 = R_4 = H (compound **11**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = neopentyl, and R_3 = R_4 = H (compound **12**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = 2-phenyl-ethyl, and R_3 = R_4 = H (compound **13**);

X_1 = X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = 2-phenyl-ethyl, and R_3 = R_4 = H (compound **14**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2= $-(CH_2)_3NH_2$, and R_3 = R_4 = H (compound **15**);

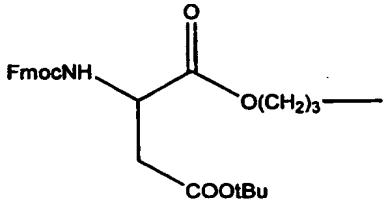
X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OSiPh_2t$-Bu, and R_3 = R_4 = H (compound **16**);

X_1 = S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OH$, and R_3 = R_4 = H (compound **17**);

X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OC(O)CH_2NH$-Cbz, and R_3 = R_4 = H (compound **18**);

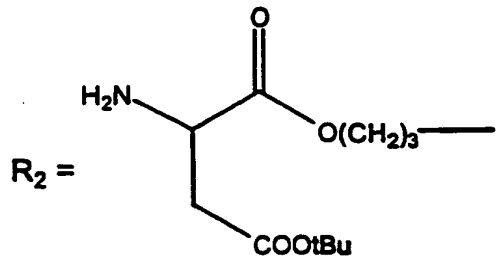
X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 = $-(CH_2)_3OC(O)CH_2NH_2$, and R_3 = R_4 = H (compound **19**);

X_1 =S, X_2 = O, R_1 = $-(CH_2)_{13}CH_3$, R_2 =



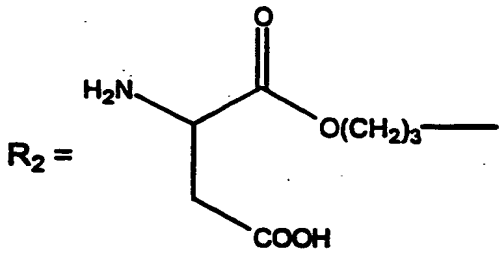
and $R_3 = R_4 = H$ (compound **20**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



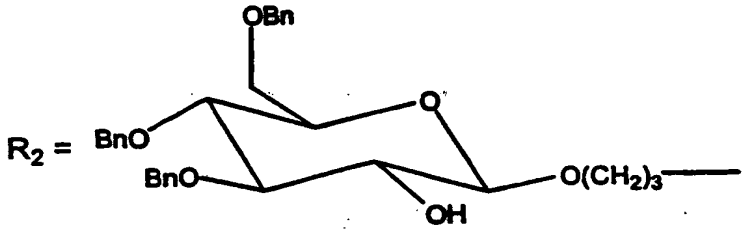
and $R_3 = R_4 = H$ (compound **21**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



and $R_3 = R_4 = H$ (compound **22**);

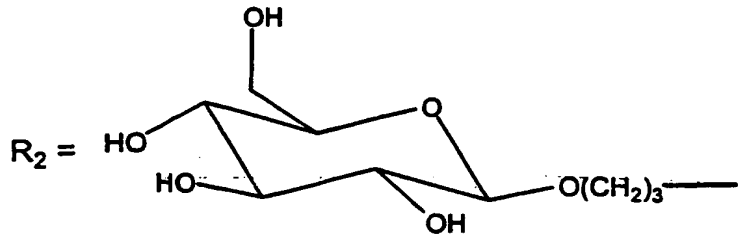
$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



wherein Bn is benzyl and $R_3 = R_4 = H$ (compound **23**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, $R_3 = -CH_2COOC_2H_5$, and $R_4 = H$ (compound **24**);

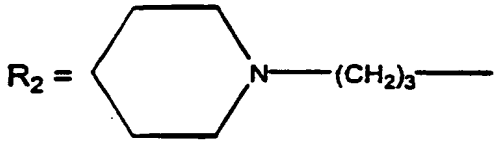
$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



and $R_3 = R_4 = H$ (compound **25**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, $R_2 = -(CH_2)_3Br$, and $R_3 = R_4 = H$ (compound **26**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$



and $R_3 = R_4 = H$ (compound **27**);

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, $R_2 = -(CH_2)_3N(C_2H_5)_3^+Br^-$, and $R_3 = R_4 = H$ (compound **28**).

3. The compounds of general formula (I) according to claim 1, where:

$X_1 = S$, $X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ (compound **3**);

$X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = ethyl, and $R_3 = R_4 = H$ (compound **4**);

$X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = *n*-propyl, and $R_3 = R_4 = H$ (compound **6**);

$X_1 = X_2 = O$, $R_1 = -(CH_2)_{13}CH_3$, R_2 = *i*-butyl, and $R_3 = R_4 = H$ (compound **10**).

4. Pharmaceutical preparations including as their active ingredient at least one of the compounds of the general formula (I) described in claims 1-3, and/or their pharmaceutically acceptable derivatives or salts, together with excipients and/or

diluents.

5. Use of the compounds of the general formula (I) described in claims 1-3 for the preparation of pharmaceutical formulations.

6. The use according to claim 5, for the preparation of pharmaceutical formulations for use in the treatment of tumours.

PATENT COOPERATION TREATY

PCT

14

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
1999PTWO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/07023	21/07/2000	22/07/1999
International Patent Classification (IPC) or national classification and IPC C07D239/54		
Applicant BRACCO IMAGING S.P.A. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19/02/2001	14.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Timmermans, M Telephone No. +49 89 2399 8940

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/07023

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-28 as originally filed

Claims, No.:

1-6 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-6
	No:	Claims
Inventive step (IS)	Yes:	Claims 2-3
	No:	Claims 1, 4-6
Industrial applicability (IA)	Yes:	Claims 1-6
	No:	Claims

2. Citations and explanations
see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents are relevant:
D1: WO 96 20710 A
D2: WO 98 52948 A
D3: EP 0714658 A

1. The novelty of the subject-matter of the present application is acknowledged. (Art. 33(2) PCT).

The compounds which are the object of the present application differ from the those described in the prior art (D1 and D2) by containing an isolated monocyclic backbone (hrydrogenated pyrimidine type) while D1 and D2 always relate to bicyclic compounds (see D1-D2 claim 1). The said compounds differ from the teaching of D3 at least by being heterocyclic compounds while D3 relates only to phenanthrene derivatives.

The subject-matter of claims 1 to 6 is considered as being novel.

2. The subject-matter of claim 1 is considered as lacking an inventive step (Art. 33(3) PCT).

2.1 Document D3, which is considered to represent the most relevant state of the art, discloses phenanthrene derivatives able to induce apoptosis from which the subject-matter of claim 1 differs in that all the claimed compounds are monoheterocyclic ones.

The technical problem underlying the application can thus be seen as the provision of further compounds able to induce apoptosis.

2.2 In view of the chemical differences existing between the compounds of the application and those described in the available prior art, it was not obvious that the said compounds would have the desired apoptosis-inducing activity.

An inventive step could thus be acknowledged for the compounds which were shown to possess the desired activity (i.e. the compounds of the examples, see page 28). Claims 2 and 3 are thus considered as involving an inventive step.

2.3 However, it must be reminded that the breadth of the claims should be such that it represents a reasonable generalization over the examples provided, and such that every compound falling within its scope actually provides a solution to the problem underlying the invention.

If it is clear that some degree of generalisation in the light of the examples must be allowed for, generalisation is taken to mean that if an example has the desired activity it can be assumed that **structurally close compounds** can be assumed to have the same qualitative activity.

The terms in claim 1 such as "aromatic", "aminic",.."ethereal groups", "aminoacids", "saccharidic portions".. do not possess a generally accepted and **limited** meaning. The said terms encompass any chemical fonction, from i.e. phenyle to any molecule or even macromolecular system which contains such a phenyle group. Those embodiments cannot be considered as obvious generalization of the examples. They were also not shown to be technical solution to the problem underlying the application.
Claims 1 and 4-6 are thus considered as lacking an inventive step.

3. The industrial applicability of the present application is acknowledged (Art. 33(4) PCT).

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
1999PTWO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/07023	21/07/2000	22/07/1999
Applicant		
BRACCO S.p.A.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. –

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/07023

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D239/54 C07D239/56 C07H15/26 A61K31/505 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D C07H A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 20710 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 11 July 1996 (1996-07-11) the whole document	1,4-6
A	WO 98 52948 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 26 November 1998 (1998-11-26) the whole document	1,4-6

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 January 2001	01/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Beslier, L

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/07023

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9620710 A	11-07-1996	US 5843943 A	01-12-1998
		AU 717243 B	23-03-2000
		AU 4601996 A	24-07-1996
		CA 2208580 A	11-07-1996
		EP 0801568 A	22-10-1997
		JP 11502193 T	23-02-1999
WO 9852948 A	26-11-1998	AU 7489698 A	11-12-1998
		EP 0986561 A	22-03-2000